STUDY OF 'T' AND 'B' CELL IN LEPROSY PATIENTS WITH AND WITHOUT REACTIONS

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RITA SAXENA

CERTIFICATE

This is to certify that the work in connection with thesis "STUDY OF 'T' AND 'B' CELL IN LEPROSY PATIENTS WITH AND WITHOUT REACTIONS" for M.D. (Pathology) of Bundelkhand University was conducted in the Department of Pathology by Dr. RITA SAXENA under my guidance and supervision. The techniques embodied in the thesis were undertaken by the candidate herself and observations recorded have been periodically checked by me.

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I N T R O D U C T I O N

INTRODUCTION

Leprosy is one of the most challenging disease known today, ranking cencern in its damage and lack of its adequate knowledge but even more challenging because of what leprosy does to its victims physicially, socio-economically and psychologically.

It is a disease of great antiquity. The leper has for centuries been a social out case, partly, from biblical times. He was regarded as unclean and partly because his repulsive appearance and disabilities prevented him from being an acceptable member of the community.

Hatred and ostracism cause concealment of the disease on the part of patients until it becomes too obvious. In doing so, the sufferer unintentionally helps to exaggerate the disease in himself as he remains without any treatment. Consequently disease takes lesser time in becoming acute enough to manifest itself too obviously in the patient. Even by this time he would have infected many other persons in his community.

The total number of leprosy cases in the world are estimated to be approximately 12 million. The figure for India being about 4.0 million patients. It is widely

distributed in all parts of this country. Causative organism of this disease is Mycobacterium leprae bacilli which was recognised by Hansen in 1874.

Leprosy involves the skin, peripheral nerves and nasal mucosa but is capable of infecting any tissue or organ. Clinically leprosy is manifested in two main clinical forms, lepromatous leprosy and tuberculoid leprosy and these two types represent the opposite poles- lack of resistance and presence of resistance in host respectively. Thus realising the importance of host immune status, an immunological approach may help in proper pathogenesis, diagnosis, control and prevention of leprosy. Ridley and coworkers (1966) have provided nomenclature, in the form of a system of diagnostic classification that is fundamental to most current immunological investigations.

As an elaboration of polar concept. Ridley and Jopling (1966) first proposed a system of five membered classification. They retained the traditional tuberculoid pole (TT), lepromatous pole (LL) and borderline (BB) group, but added two intermediary categories, borderline with tuberculoid features (BT) and borderline with lepromatous features (BL) - TT, BT, BB, BL, LL, thus, comprise a spectrum in continuity. They also explained that each stage in spectrum was determined by the result of host

response to antigen of Mycobacterium leprae. Patients with BL have more immunity against Mycobacterium leprae than do the LL but less than patients with BT and TT. So it indicates that TT patients have highest and LL have lowest immunity.

The tuberculoid type of leprosy is seen in patients with high degree of resistance. The skin lesions are few and sharply demarcated, consisting of macular anaesthetic patches. Nasal involvement occurs early and may be profound leading to deformities particularly of hands and feet. Bacilli are scanty in lesions and infectivity is minimal, cell mediated immunity is inadequate and lepromin test is positive.

The lepromatous type host resistance is low. The bacilli are seen in large numbers on globi inside lepra cells or extra cellular superficial nodular lesion. Bacilli invade the mucosa of nose, mouth and upper respiratory tract. The eyes, testes, kidney and bones are also involved. The lepromatous type is more infective than others. Cell mediated immunity is deficient and lepromin test is negative.

Present study has been undertaken to assess the cellular immunity in different types of leprosy patients. The tests, which were taken in assessment, were status of T-cell and B-cell in peripheral blood.

It has been observed that leprosy patients commonly get the acute exacerbation occuring in the otherwise chronic course of the infection. This sudden spurt is quite distinct from the normal progression of the disease process. This sudden exacerbation of the disease is designated as reaction. The T and B cell levels in leprosy reaction cases and leprosy non reaction cases have been also assessed to signify the role of immunity in reaction in various types of leprosy.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

INTRODUCTION AND HISTORY

Leprosy is known since antiquity, but where it took it's origin remains obscure. Possibly this disease started in Africa and spread through out the world including India via routes of pilgrimage and travel (Scott, 1943). In "Shushrut Samhita" (600 BC), leprosy has been described as "Vat rakt" or "Vat shonita" and "Kushta". Though these conditions probably included psoriasis and vitiligo, leprosy was certainly described. "Kushta" was also mentioned in Vedas and the disease leprosy in our country is present at least since 1400 BC, if the contention of Roger and Muir (1940) and Lowe (1942) is correct.

In European countries this disease was recognised from second century A.D. (Anderson, 1969). One finds a reasonably good account of leprosy at several places in Bible but it is doubtful whether the word has reference to the same disease what we know today as leprosy. (Lie, 1938; Lendrum, 1952). "Zaraath" in Jewish and 'Lepra' in Arabic literature stand for scaly and fungal diseases. The term Zaraath in old test-a-ment and term'lepra' in new test-a-ment has been considered by many authors to refer to leprosy, and in all Biblical translations has been rendered as leprosy.

The exact number of leprosy patients around the world is not definite. WHO estimated leprosy patients to be over 12 millions including 4 millions in Africa, 7 millions in Asia and about 0.5 millions in America. In India 1971 census showed it to be 3.2 millions, and 1981 census about 4 millions. Uttar Pradesh is moderately endemic state with about 0.05 millions of estimated cases.

IMMUNOLOGY OF LEPROSY

Leprosy is an infectious disease caused by Mycobacterium leprae (M. leprae). It presents itself in the form of a clinical, pathological, bacteriological and immunological spectrum (Ridley and Jopling, 1966; Skinsnes, 1973). At one end of the spectrum is the tuberculoid (TT) leprosy with high resistance and strong delayed hypersensitivity. At the other pole of the spectrum is a low resistance form the lepromatous leprosy, characterised by multiplicity of lesions abounding in Mycobacterium leprae.

GENETIC FACTORS

The host resistance can vary because of various interacting factors including constitutional and environmental. Earlier circumstantial evidence suggested that it was the host factor variation rather than existence of different types of disease. Varied forms of leprosy occur in the same family

where strains of Mycobacterium leprae, are expected to be the same (Newell, 1966). Secondly, there is high incidence of concordance for leprosy in monozygotic twins than in dizygotic twins (Ali, 1966; Chakraborty and Vogel, 1972). Lack of variation in the virulence of different strains of lepra bacilli was confirmed by Rees (1969). He demonstrated that leprosy bacilli from patients with different types of leprosy all behave in the same way when injected into susceptible mice.

A number of other studies, Viz., taste sensitivity to phenyl thiourea (Beiguelman and Marques, 1964), correlation with A.B.O. blood group system (Hsuen, Thomas and Jesudian, 1963; Lechat et al, 1968), glucose-6-phosphatase dehydrogenase deficiency (Beiguelman, 1966), the frequency of Australia antigen (Blumberg and Melartin, 1967; Samuel, Samuel and Godal, 1974; Bedi, Sama and Bhutani, 1975) and the distribution patterns of HLA antigens (Godal and Myrvang, 1973; Kreisler et al, 1974; Das Gupta et al, 1975; Smith et al, 1975) have been done to assess the role of genetics in leprosy. These studies have given conflicting reports and the role played by genetics in leprosy even today, remains controversial.

HUMORAL IMMUNITY

Mycobacterium leprae is an intracellular parasite. It is well known that the circulating antibodies can not eliminate intracellular parasites (Mackaness, Blanden and

Collins, 1966). The intracellular organisms, therefore, require cell-mediated immune (CMI) mechanism for the control of infection (Mackaness et al, 1966). The variation in the host resistance to M.leprae is thus related to cell-mediated rather than the humoral antibody response which may in fact be enhanced in lepromatous (low resistance) form of the disease. Various types of immunoglobulins in the sera of leprosy patients have been shown to be raised by various workers. Much more higher levels were demonstrated among lepromatous than the tuberculoid (Uhr and Moller,1968; Bullock, Ho and Chen, 1970; Jha et al.,1971; Young chaiyud et al., 1975). Non specific mycobacterium antibody against protein antigens of M.leprae have been shown to be raised in lepromatous leprosy. (Abe et al., 1972). The production of antibodies to unrelated antigens such as typhoid and paratyphoid appears to be normal in both types of leprosy (de Almeida, Bandao and de Lima, 1964; Sheasgren et al., 1969; Jha et al., 1971). A number of autoantibodies have also been demonstrated in the sera of patients with lepromatous leprosy (Matthews and Trautman, 1965; Bonomo and Dammacco, 1968; Wager, 1969; Saha and Mittal, 1972; Rea et al., 1974).

ANTIGENIC CHARACTERIZATION OF M.LEPRAE

Characterization of Mycobactrium leprae antigens and demonstration of specific anti M. laprae antibody

remained dificult till recent time. Harboe et al. (1978) carried out immunochemical characterization of M. leprae by crossed immune electrophoretic analysis but failed to identify any specific antigen. All the seven antigens which they had isolated from M. leprae cross reacted very strongly with other mycobacteria. With the newer methods like iso electric focussing, various protein antigens have been identified. Navalkar (1984) demonstrated that only M.leprae bands extended to pH 6.5 while other mycrobacterial bands were seen between pH 4 and 5 on polyacrylamide gel plates. Similarly phenolic glycolipid antigen of M.leprae have been found to be specific (Brett, 1984). Other recent developments, the monoclonal antibody and the enzyme linked immuno assay (ELISA) helped (Sengupta and (Sinha, et. al. 1983). It has been shown that monoclonal antibodies recognised specific and non specific epitopes of M. leprae antigens (Ivanji et al.,1983) and also one of the monoclonal antibodies (ML04) has been used for an specific serological assay for the detection of M.leprae antibody (Sinha et al., 1983). ML06 is another specific monoclonal antibody. Recently Narayana et al.(1983) demonstrated both these monoclonal antibodies in both tuberculoid and lepromatous granulomas while no staining with these antibodies could be obtained in lesions of tubercular lymphnode, psoritic skin or normal skin. Further, the M.leprae in lepromatous lesions also could not be stained. The lymphocytes and macrophages in both the tuberculoid and lepromatous

granulomas showed membranous staining with the above antibodies.

ROLE OF CELL MEDIATED IMMUNITY

Cell mediated immunity can be assessed by a number of in-vivo and in vitro parameters. Lepromin and tuberculin skin reactivity, ability to develop contact sensitivity, survival of skin homografts, architecture of lymphnodes and ratio of B and T cells all help in the study of CMI responses. CMI can also be assessed by in vitro tests like lymphocyte response to various mitogens and antigens and production of lymphokines (MIF) by lymphocytes.

LEPROMIN REACTIVITY

Some of the earliest studies to assess the immune status of leprosy patients were understandably concerned with the skin reactivity to lepromin. Lepromin skin reaction is reported to parallel the host resistance to M. leprae (Mitsuda, 1953; Hayashi, 1933) and are therefore negative in BL, LI and LL leprosy; moderate in BB and BT and strongly positive in TT leprosy (Ridley and Jopling, 1966). Talwar et al, (1972) have studied the lepromin test in the spectrum of leprosy and they found that both early and late reactions were negative in LL but early reaction was positive in some BL. Further, BB patients had only positive early reactions while late reaction was negative. In TT and BT patients

both early as well as late reactions were found to be positive. Other workers have noted similar observation (Bedi et al., 1976; Job et al., 1976; Rea et al.,1976; Sharma et al., 1979; Kumar et al., 1980; Rao and Rao, 1981; Singh et al.,1985).

The lepromin negativity in lepromatous leprosy is more or less specific (Turk and Brycesson, 1971; Chatterjee, 1976). In lepromatous patients non-specific depression of responses to other intradermal antigens (tuberculin, trichophytin and odiomycin) has also been reported (Rotberg, 1938; Lima and Mogaroa, 1962; Bullock, 1968; Waters, 1971; Mendes et al., 1974). Lowe and Mc Nulty (1953) however, did not find difference in tuberculin reactivity among lepromatous and tuberculoid patients. Rea et al,(1974) using streptokinase/ streptodornase, mumps antigens, trichophytin, candida antigens or histoplasmin also failed to find any difference between lepromatous leprosy patients and a control group of dermatological patients.

CUTANEOUS HYPERSENSITIVITY

The reduced capacity in lepromatous leprosy patients to develop cutaneus hypersensitivity to such chemical sensitizers, as picryl chloride and dinitrochlorobenzene (DNCB) also reflects a non specific depression of the host's CMI. Most of the workers (Waldorf et al.,1966;

Turk and Waters, 1969; Saha and Mittal, 1971) have shown a lowering in the rate of sensitization to DNCB and picryl chloride in leprosy as compared to normals. The depression is of greater magnitude among lepromatous leprosy patients and less in the tuberculoid patients. However, Mendes et al,(1974) and Rea et al,(1974) observed near normal contact sensitivity to DNCB among lepromatous patients. Both Waldorf et al,(1966) and Bullock (1968) observed a lack of correlation between the impaired ability to develop contact sensitivity and tuberculin reactivity. This lack of correlation suggested that failure to chemical contact sensitivity probably detected only a partial failure of CMI was further evidenced by the work of Turk and Waters (1969), who were able to induce sensitivity to key-hole-limpet hemocyanin (KLH) in all the nine lepromatous sensitized with DNCB.

GRAFTS

In-vivo, prolonged survival of skin homografts is regarded as a non-specific indicator of cell mediated immunity depression. Prolonged survival of skin homografts has been observed in lepromatous patients (Job and Karat, 1967; Han, Weiser and Kau, 1971). Han, Weiser and Kau (1971) noticed a similar though less pronounced survival of the graft in tuberculoid patients. Ptak et al,(1970) reported a prolonged survival of skin allografts in mice infected with M. Lepraemurium.

ARCHITECTURE OF LYMPHNODES

Another change which may be seen in vivo with a depression of cell mediated immunity is the depletion of lymphocytes from paracortical areas of lymphnodes. Such changes are seen in Wiskott-Aldrich syndrome a disease with decreased CMI (Cooper et al., 1968) and in animals treated with antilymphocyte serum (Turk and Willoughby, 1967). Desiken and Job (1966) and Turk and Waters (1968, 1971) reported similar finding in lymphnodes among lepromatous leprosy patients; the lymphocytes were replaced by bacilli laden macrophages. Turk and Waters (1971) further observed that the hystiocytes became epitheloid along with an increase in number of lymphocytes in paracortical areas as resistance to infection increases across the leprosy spectrum. Ptak et al, (1970) found a similar depletion of paracortical areas of lymphnodes in mice infected with M.lepraemurium.

LYMPHOCYTE TRANSFORMATION (LTT)

Various in vitro parameters corroborate the in vivo findings of depressed CMI in lepromatous leprosy. A severe depression of phytohaemagglutinin (PHA) induced lymphocyte transformation in lepromatous leprosy has been demonstrated (Rodriguez- Paradisi, de Bonaparte and Morgenfeld, 1967, 1968; Bullock and Fasal, 1968, 1971; Dierks and Shepard, 1968; Han, Weiser and Lin, 1972; Yamada and Fuzimoto, 1974). In tuberculoid leprosy also, some of the

studies suggest that the lymphocyte response may be depressed though this is neither that pronounced nor consistant (Bullock and Fasal, 1968, 1971; Dierks and Shepard, 1968; Han, Weiser and Lin, 1971; Wong et al, 1971). While some authors (Sheagren et al, 1969; Nelson et al, 1971; Ulrich et al, 1972; Jacob et al, 1974; Rea et al, 1974) failed to demonstrate a depression of lymphocyte response to PHA in lepromatous leprosy also, the evidence in favour of depressed lymphocyte response to PHA is sufficiently convincing. Further studies by Kaklamanis et al,(1977) have revealed that the depressed response to PHA was associated with the reduction in circulating T-lymphocytes. Other workers also observed the same response (Rea et al, 1976; Nath et al, 1977; Sharma et al, 1979; Ghei et al, 1980; Dubey et al, 1981).

Other non-specific mitogens- pokeweed mitogens, and antigens - streptolysin - 'O' and PPD have given similar results (Sheagren et al, 1969; Bullock and Fasal, 1971; Talwar, 1972; Ulrich et al, 1972). All these mitogens and antigens show a non-specific depression of CMI.

The lymphocyte response to specific M.leprae antigens is also significantly depressed (Bullock and Fasal, 1971; Godal et al,1971; Talwar et al, 1972; Myrvang et al, 1973). A continuous decrease in lymphocyte response

to M.leprae from the TT to LL end of the spectrum by both morphological and tritiated thymidine uptake methods had been reported (Myrvang et al, 1973).

LYMPHOCYTES (T AND B)

From the above it is abviously clear that humoral immunity is intact and CMI is impaired among leprosy patients, more so among lepromatous group. Lymphocytes are involved in immunological mechanism (Harris et al,1945). The thymus dependent (T-cells) appear to be concerned with CMI and bursa dependent (B-cells) with the humoral immunity (Clamen et al,1966; Roitt et al, 1969; Greves et al, 1973). Thus assessment of T-cells status is important while considering CMI.

Various workers have shown that the T-cell count in the peripheral blood had decreased gradually in the spectrum of leprosy from tuberculoid pole to lepromatous pole; maximum fall being in lepromatous and minimum being in tuberculoid pole; contrary to this B-cell count is found to be increased towards lepromatous pole than tuberculoid pole (Dwyer et al, 1973; Gazlpeczalska et al, 1973; Lim et al, 1974; Chogle et al, 1977; Sharma et al, 1979; Sachdev et al, 1980). In contrast, in the tuberculoid type of leprosy the number of E-rosetting T-cells remain at normal level (Nath et al, 1973; Manimakalai et al, 1982).

Similarly in the lymphnodes of lepromatous leprosy patients, it has been demonstrated that number of B-cells is high in comparision to B-cells in lymphnodes of normal human beings (Verma et al, 1971). This is similar to what one finds of B-cells in peripheral blood of leprosy patients.

Mendes et al, (1974) have studied T and B cells in peripheral blood as well as in lymphnode of lepromatous leprosy cases. A significant decrease in proportion of T-cells was observed in peripheral blood and depletion of T-cells in para cortical areas of involved lymphnodes indicating impaired CMI. B-cells were found to be increased in peripheral blood as well as preservation of B-cells areas was seen in lymphnodes. Similar observations have been made by Methias et al (1980) in the study of cellular changes in spleen.

Studies of infiltrating cells in cutaneous lesions of leprosy have shown similar findings. Kaplan et al, (1984) demonstrated that cutaneous infiltrates of patients of lepromatous leprosy (LL and BL) contained only small numbers of scattered lymphocytes, mostly of the Leu 2a/ OKT8 T-cells subset. In borderline patients (BL and BB) and increase in the number of lymphoid cells specifically of the Leu 3a/OKT4 T-cells subset was observed. At the tuberculoid pole of the spectrum (BT and TT), large

numbers of T-cells with extremly long and complex filipodia were found closely associated with epitheloid cells and multinucleated giant cells.

CMI SUPPRESSION

The failure of CMI in leprosy, more so among lepromatous leprosy patients has been explained either because of suppressor cells (suppressor T-cells), serum factors of impaired macrophage functions. A number of workers (Bullock et al, 1968; Nelson et al, 1971; and Jaswaney et al, 1982) demonstrated the presence of suppressor factor(s) clearly in lepromatous serum. Jaswaney et al,(1982) have shown that lowering in the numbers of T-cells in lepromatous leprosy can reverse to normal levels by incubating the lymphocytes in foetal calf serum (FCS). However, Patel et al,(1984) could not obtain this stimulatory effect of foetal calf serum on the number of T-cells after 24 hours of incubation. Further,when FCS treated cells were incubated for another 24 hours for rosetting in normal healthy serum, tuberculoid (TT/BT) serum, lepromatous (BL/LL) serum respectively, a very stimulatory effect was noted in T-cells numbers in all the sera. It has been amply demonstrated that such "suppressor" factor in the serum of lepromatous patients comes from infected macrophages (Salgame et al, 1984; Satish and Nath, 1983). This factor initiate suppressor function of lymphocyte transformation tests (LTT) to both

specific M.leprae mediated antigen stimulation and non specific (con A stimulation) mitogenes.

As mentioned earlier the unresponsiveness of lepromatous patients to antigens of lepra bacillus, and there partial responsiveness to related antigens for example tubercle bacillus, would be due to the presence of a specific population of suppressor lymphocytes capable of being triggered by at least oneunique antigen of M.leprae. Mehra et al, (1980, 1982, 1984) have shown that : (i) Dharmendra lepromin induced in vitro suppression of the con A response of lepromatous and borderline, but not tuberculoid patients or normals (ii) two cells populations contribute to the suppression, monocytes and T-cells (iii) all the lepromin induced T cells activity was associated with a 20-30% sub-population of T-cells defined by the thymidine, monoclonal OKT5 or OKT8 antibodies (iv) a high percentage of the T-cells subset expressed activation markers, Ia and Fc receptors (v) some T-cells recognised the unique phenolic glycolipid I of M.leprae, which could induce supression of mitogenic responses in vitro as well as lepromin and (vi) no significant suppression in vitro was found with lymphocytes from 60 lepromatous patients after immunotherapy with BCG + killed M.leprae, and the number of a Ia + OKT8 + cell returned to normal levels.

In this context it is interesting to note that Salgame et al,(1984) have shown that exposure of suppressor factor to T-cell make them as suppressor cells by altering membrane of T-cells, since colchicin treatment reverses the suppressor activity of T-cells.

MACROPHAGES-STATUS IN LEPROSY

Role of macrophages in CMI is mentioned earlier- for effective CMI the co-operation of antigen presenting cells and responding lymphocytes is essential. The macrophages process the antigen and present it to the lymphocytes. They also produce factors (Monokines) which may stimulate or inhibit the lymphocytes and may have cyto toxic properties also (Mackaness, 1969 and Territo and Clind, 1975).

The functional status of macrophages has been investigated. In 1967, Barbieri and Correa reported that macrophages from Mistuda - Negative individuals were inactive in vitro against M.leprae, while macrophages from Mistuda - positive persons caused the haemolysis of bacillus in vitro. Similar results have been observed by Beiguelman (1967) and Pisani et al,(1973). Recently Sharp, Calston and Benerjee (1985) showed that M. leprae are not killed by peritoneal macrophages of Nude mice. However, macrophage activity (killing) parallels infection in normal mice. There appears to be defective macrophage population in lepromatous patients that is unable to process M.leprae antigen and initiate the CMI response.

M.leprae on entering into the macrophages of "susceptible" individual produce product(s) that alter metabolism of host cells and also alter the membrane chemical architecture of the cells. Salgame et al, (1980) showed that entry of M.leprae into the macrophages of lepromatous patients, reduced the level of protein systhesis of the host. This has been confirmed to be true even for lysosomal enzymes (Marolia and Mahadevan, 1984). Birdi et al, (1980) demonstrated the occurence of membrane charges in "susceptible" macrophages by the entry of live M.leprae by monitoring Fc receptors, HLA Dr-antigens. Similar changes have been demonstrated by monitoring con A receptors (Salgame et al, 1983). Lad et al,(1983) have done the same with the adherence receptors that enable M.leprae to adhere to the cell.

Birdi et al,(1980) also demonstrated that there is an inability of the macrophages from leprosy patients to undergo antigen mediated (dead or live M. leprae) physical intraction with lymphocytes, unlike the macrophages from the "resistant" normals or tuberculoid leprosy individuals. This is also most probably, due to membrane perturbation in the susceptible macrophages.

REACTION IN LEPROSY :

Leprosy is a chronic disease with the slow and gradual progression spread over a number of years. However, during the placid course of the disease, abrupt changes in

the clinical stability of the disease or acute outbursts of activity occur and these are termed reactions (Ridley, 1969; Bedi and Bhutani, 1975; Jollifee, 1977). These are different from mere extension or regression of disease. These acute inflammatory reactions have immunological basis.

Reactions are characterised by certain clinical, pathological, immunological and possibly bacteriological changes. Considerable confusions has, in the past, been caused by use of different terminology to describe the reactions; probably, the most misinterpreted term having been "lepra-reaction" which has variously been used to denote either the entire group of reactions or the progressive lepra reaction in lepromatous leprosy (Jopling,1971). Yet, another interpretation (Cochrane, 1964) given to lepra reaction in reactions in lepromatous leprosy only. Waters, Turk and Wemambu (1971) suggested that the majority of these reactions may be classified into two aetiological groups, type I and type II reactions. This classification has been followed by other workers and accepted generally (Jolliffe, 1977).

TYPE I REACTIONS :

This type of reaction is found in patients with nonpolar type of leprosy (BT, BB and BL) whether treated or untreated. These type I reactions may be divided into

two sub types; namely reversal or upgraded and down graded reactions depending upon the changes in the CMI. Evidence that this reaction is caused by alteration in CMI is provided by the following (1) Changes in the Mistuda reaction; (2) Altered reactivity of lymphocyte transformation tests using whole M.leprae antigen (Barnetson, Pearson and Pus, 1976); (3) Histopathological evidence in reacting skin and nerve lesions of changes in the numbers of defending cells such as lymphocytes, epitheloid and giant cells and changes in the numbers of viable bacilli (Ridley,1969); (4) Lymphnode histological evidence of changes in the cell types occupying the T-cell, paracortical areas. Polar lepromatous (LL) sub polar lepromatous (LI) and the normal lymphocyte population is replaced by macrophages full of bacteria. In reversal reactions the lymphocytes reappear, suggesting return of T-cell activity (Turk and Waters, 1971).

The type I reactions are precipitated by the sudden change in the hypersensitivity in the individual to antigens (Skinsnes, 1973). The exact mechanism is not clear. The change in CMI may be in any direction. Occasionally, however, there may be no change in the CMI. It is usually not possible to differentiate clinically the shift of the immunological status even when new lesions appear. Thus down grading reactions are associated with a decline of immunity and a corresponding increase in the number of bacilli and extension

of infection in near tuberculoid and borderline patients. By their nature these reactions are likely to be found only in untreated patients. Reversal reaction are the opposite. They occur in near lepromatous and borderline patients when the bacterial load is diminished as a result of treatment, and they are associated with a corresponding increase of immunity (Fernadez et al, 1962).

Godal et al,(1973) have also shown concomitant changes in CMI in reversal reactions through various in vitro tests viz. lymphocyte transformation test (LTT) and leucocyte migration inhibition test to M.leprae antigens. In this reversal type of reaction the number of lymphocytes is increased in the cutaneous lesions as well as in the lymphnode. Such reactions have been experimentally produced in immunologically suppressed mice with lepromatous leprosy on infusions of syngeneic lymphoid cells (Rees, 1970).

TYPE II REACTIONS :

This second type of reaction (Type II) or so called ENL reactions usually manifest itself by the end of first year of sulfone therapy in about 50% of polar and sub polar lepromatous patients and less frequently in borderline cases. Rea and Levan (1980) reviewed 32 patients with this form of reaction and less than a third of them had received any form of anti leprosy therapy prior to its

onset. They stressed that this reaction is a manifestation of the disease and not always a complication of its therapy.

The typical cutaneous and subcutaneous crops of tender erythematous papules or nodules (Erythema nodosum leprosy, ENL) may be associated clinically with rigor and fever. Sometimes this may be complicated by neuritis, arthritis, iridocyclitis, orchitis, proteinuria and lymphadenopathy. Histologically the lesions resembles, Arthus reaction. The centre of ENL lesion comprises disintegration of macrophages with release of bacterial (M.leprae) antigens. There is intense perivascular bacteria free polymorphonuclear leucocytic (PMN) infiltration along with intense vasculitis with fibrinoid necrosis in deep dermal vessels and capillary necrosis in more superficial vessels (Ridley, 1969; Job, Gude and Macadin, 1964). The bacilli are usually relatively sparse in lesions of ENL.

The ENL reactions are believed to result from a combination of mycobacterial antigens with circulating antibodies. Wemambu et al, (1969) demonstrated immunoglobulins and complements in ENL lesions. Electron microscopic studies have proved successful in localizing the M.leprae antigen in the cytoplasmic fraction which forms immune complexes with circulating antibodies (Humoral antibodies are raised in sera of lepromatous patients. Infact such circulating immune complexes have been demonstrated in sera of patients

suffering from ENL by Moran et al, (1972). Furthermore, Lin Shwe (1971) has shown that serum complement is utilized in these immune reactions. The findings of impaired fibrinolytic activity and increased serum histaminase levels in ENL patients are also in conformity with this being an Arthus phenomenon.

Recently, Mshana (1982) however, proposed that ENL is initiated by a decrease, absolute or relative of suppressor T-cells. He based this hypothesis on the following: (i) Contact sensitivity to dinitro-chlorobenzene (DNCB) is depressed in lepromatous leprosy but greatly attenuated or not at all impaired during ENL (Rea and Levan, 1980). (ii) Bach et al, (1980) using monoclonal antibodies demonstrated that there is a depression of suppressor T-cells during ENL with a concomitant increase in vitro phytohaemagglutinin (PHA) response, although vivo responses to M.leprae were not affected. (iii) Despite circulating M.leprae antibodies, not all patient develope ENL as a complication. (iv) Various factors known to precipitate ENL seem to share little in common in terms of immune complexes. In their view most of the factors associated with precipitation of ENL are causing a disturbance in the T-cell balance thus favouring a drop in the suppressor cells. The observation of Saha

et al,(1973) on ENL occuring after small pox vaccination is taken as a support suggesting that ENL in this instance is precipitated by the T-cell sub population starting with an increase in suppressor cells, as in common with viral infections, and then followed by a decrease of this population which results in ENL precipitation. Further work on this hypothesis is awaited for confirmation.

MATERIAL AND METHODS

MATERIAL AND METHODS

The study has been conducted on patients suffering from various types of leprosy, admitted or attending out patient Department of Skin and V.D., M.L.B. Medical College, Hospital, Jhansi. The patients were examined in the period between July, 1985 to March, 1986. Normal healthy volunteers without immunological disorders were selected from Department of Pathology and from relatives of patients as controls.

Patients were thoroughly examined clinically for type of leprosy and findings were recorded on a predesigned proforma (Appendix - I).

COLLECTION OF BLOOD SAMPLES

Blood samples in the quantity of 10 ml with heparin as anticoagulant were collected in sterile tubes from patients with strict aseptic precautions. Routine haematological tests were done from blood collected simultaneously from each patient.

SKIN BIOPSY

Biopsy material was excised from the edge of the lesion and collected in 10% formal saline. It was dehydrated in serial concentrations of alcohol and cleaned

with xylene in place of chloroform. Tissue was embedded in paraffin (58°-60°C), blocks prepared and sections of 5 micron thickness were cut by rotatory microtome. Staining was done with haematoxylin-eosin. For demonstration of lepra bacilli modified Fite-Faraco staining was done.

HISTOPATHOLOGICAL TYPING

Criteria of Ridely and Jopling (1966) as given below was adopted for histopathological typing of leprosy cases.

TUBERCULOID TYPE (TT)

- Foci of well developed epitheloid cell granuloma with a few Langhan's giant cells, often enveloped by dense zone of lymphocytes, especially in deeper parts of dermis.
- Erosion of basal layer by granuloma.
- Nerves difficult to detect and may show caseation.
- Lepra bacilli not detectable by modified A.F.S.

BORDERLINE TUBERCULOID (BT)

- Narrow clear subepidermal zone above the granuloma.
- Lymphocytes plentiful and diffuse.
- Nerves swollen but recognisable.
- Lepra bacilli demonstrable 0 to ++.

BORDERLINE (BB)

- Sheets of epitheloid cells but no giant cells.
- Lymphocytes sparse and diffuse.
- Nerves showing structural disorganisation but no granuloma.
- Lepra bacilli demonstrable in grade +++ to ++++.

BORDERLINE LEPROMATOUS (BL)

- Histiocytic granuloma with cells slightly epitheloid in appearance.
- Few diffuse lymphocytes.
- Nerves swollen or normal.
- Lepra bacilli demonstrable in grade ++++ to +++++.

LEPROMATOUS LEPROSY (LL)

(a) Active phase

- Macrophages with some foamy changes.
- Very scanty lymphocytes.
- Nerves almost normal.
- Lepra bacilli +++++ to ++++++.

(b) Regressing phase

- Foam cells with globi and much fat.
- A few lymphocytes
- Lepra bacilli ++++ to +++++.

REVERSAL REACTIONS

- Influx of lymphocytes
- Oedema in and around granuloma.
- Granuloma increases in volume.
- Cytological changes more towards epitheloid form.
- Number of bacilli gradually diminished.
- Foreign body giant cells present.
- In severe cases there is necrosis.
- In a patient having erythema and swelling of skin lesion often there is nerve involvement new lesions may appear. They may present tuberculoid appearance.
- Lymphocyte decrease in number.
- Granuloma spreads.
- Large giant cells of foreign body type appear.
- Cells are vacuolated (Intracellular oedema) and extracellular oedema is a constant finding.
- Bacilli appear in detectable number.

EXACERBATION NODULES

- One lesion becomes exceptionally large and loaded with many times more bacilli.
- There is healthy polymorphonuclear infiltration and cellular disintegration.

- Polymorphs are present.
- Cellular disintegration is marked.
- Later there may be significant number of lymphocytes.
- Vasculitis or vascular necrosis is prominent in some.
- In a patient having crops of small painful red nodule with lymphnode, liver, spleen enlargement and iridocylitis,orchitis and painful enlargement of nerve seen. Temperature found to be raised.

BLOOD EXAMINATION

The method of Dacie and Lewis (1975) was followed for total and differential leucocyte counts. From total and differential leucocyte count, absolute lymphocyte count was calculated using the following formula.

$$\text{Absolute lymphocyte count} = \frac{\text{Total Leucocyte Count} \times \% \text{ of lymphocytes}}{100}$$

$$\left(\text{A L C} = \frac{\text{T L C} \times \% \text{ LYMPHOCYTE}}{100} \right)$$

EVALUATION OF T AND B LYMPHOCYTE

T and B lymphocytes present in the peripheral blood were demonstrated by means of their surface receptors (Jondal et al, 1972).

The basic principle of procedure is as follows:-

I. Separation of lymphocytes.

II. Demonstration of T cells by sheep red blood cells (SRBC) rosette (E rosette).

III. Demonstration of B cells by formation of rosettes with SRBC coated with antisheep haemolysin antibody and complement (EAC rosette).

LYMPHOCYTE SEPARATION

The lymphocytes were separated by Ficoll Conray 420 density gradient centrifugation. The separation of the lymphocytes by this method was due to differences in the density of various cells in the blood.

MATERIAL REQUIRED

1. Ficoll Conray 420 solution (specific gravity 1.077) was prepared as described by Sen Gupta (1981). (11.40 gm of Ficoll dissolved in 160 ml of distilled water + 22 ml of Conray 420. Specific gravity was adjusted to 1.077 and sterilized by Seitz filter).
2. Preservative free heparin.
3. Minimum essential medium (Eagle) with Hanks base (Micro Lab., Bombay).
4. TC Medium 199 (Difco Laboratories, Detroit Michigan, USA).

5. PHA-M (Difco Laboratories, Michigan, USA).

6. Alsever's solution :-

Glucose	....	24.6 gm
Trisodium Citrate(Dehydrate)	..	9.6 gm
Sodium Chloride	....	50.04gm
Distilled water	....	1200 ml

pH was adjusted to 6.1 with 10% citric acid.

This was sterilized in autoclave under low pressure.

7. Phosphate Buffer Saline (PBS)

(A) Phosphate buffer solutions

0.15 M - NaH_2PO_4, $2H_2O$ 23.4gm/Litre in Distilled water.

(B) 0.15 M - $Na2HPO_4$ 21.3gm/Litre

(C) Normal saline (NaCl) 9 gm/Litre

The pH of phosphate buffer saline was adjusted to 7.4 by mixing solution 'A' 18 ml

Solution 'B' 82 ml

Normal saline 100 ml was added to prepare phosphate buffer saline.

8. Glutaraldehyde 2 % solution in PBS

9. AB sera collected from persons of AB blood group.

10. Complement from guinea pig.

METHOD

I. 2.5 ml Ficoll solution was taken into two sterilized test tubes.

II. 5 ml of blood was poured in each test tube carefully in such a way that a layer of blood is formed. It was centrifuged immediately at 1500 rpm for 15-20 minutes.

III. After centrifugation various layers are formed which contain plasma, predominantly lymphocytes, Ficoll Conray, neutrophils and red cells from upper to lower layer respectively.

IV. After removing plasma carefully the lymphocyte layer is pippetted off and placed in 5 ml of MEM in one test tube and 5 ml of TC medium 199 in the other and washed twice, centrifuging each time at 1000 rpm for 10 minutes with MEM and TC medium, respectively.

V. The cells were suspended in MEM and viability was checked by 1% eosin in TC medium 199 and count was adjusted to 2-3 X 10^6 cells/ml.

(B) DEMONSTRATION OF T CELLS (SRBC ROSETTE FOR T CELLS)

1. Sheep red blood cells (SRBC) were collected in Alsever's solution and washed thrice in phosphate buffer saline (PBS) and suspension made up to 0.5% in PBS.

2. 0.5 ml of lymphocyte suspension was mixed with 0.5 ml of SRBC and incubated for 15 min at 37°C.

3. Then it was kept at 4°C overnight.

4. 1 ml of 2% glutardehyde was added and test tube was kept in ice for 15 minutes.

5. Finally the wet preparation was made and stained with 0.2% methylene blue and 200 cells were counted.

Three or more SRBC adhering to lymphocytes were taken as rosette forming cells. The absolute T-cell count was calculated as below :-

$$\text{Absolute T cell count} = \frac{\text{ALC X \% of T cells}}{100}$$

(C) DEMONSTRATION OF B CELLS (EAC ROSETTE FOR B CELLS)

1. SRBC were washed thrice with PBS and adjusted to a concentration of 5%.

2. 0.5 ml of 5% SRBC was added to 0.5 ml of antisheep haemolysin (Amboceptor) and incubated for 15 minutes at 37°C. Subhaemolytic dose of amboceptor was assessed before putting the test (Cruickshank, 1975).

3. SRBC were washed thrice with PBS and suspended with 0.5 ml of PBS.

4. 0.5 ml of 1:10 dilution of complement guinea pig added to SRBC and incubated for 45 minutes at 37°C.

5. These SRBC (ie EAC now) were washed thrice with PBS and suspended to make 0.5% concentration in PBS.

6. 0.5 ml of lymphocytes were added to 5 ml of 0.5% of EAC in PBS and incubated at $37^{o}C$ for 30 minutes.

7. The solution was suspended and wet preparation was prepared and stained with 0.2% methylene blue and then 200 cells were counted.

Three or more SRBC adhered to lymphocyte were considered to be rosette.

Absolute B cell count was calculated as follows :-

$$\text{Absolute B cell count} = \frac{\text{ALC X \% B cell}}{100}$$

O B S E R V A T I O N S

OBSERVATIONS AND RESULTS

The present study was undertaken on 60 subjects out of which 45 were various types of leprosy patients and 15 were nonleprotic volunteers who served as controls (Table I).

TABLE I

DISTRIBUTION OF CASES

Groups	No.of cases
Control cases	15
Study group cases	45

The control group included healthy relatives of patients admitted to this hospital and employees of the department of Pathology. The controls were not receiving any drug or suffered in recent past with any illness which might have affected their immunological status.

STUDY GROUP CASES

The study group cases were selected from patients admitted or attending out patient department of Skin and V.D. They were distributed on the basis of

clinical examination as shown in Table II. Out of 45 cases, 11 were of tuberculoid type (TT), 5 were of borderline tuberculoid type (BT), 11 were of borderline (BB), 5 were of borderline lepromatous (BL) and 13 were of lepromatous type (LL), (Table II).

TABLE II

DISTRIBUTION OF CASES ON CLINICAL EXAMINATION

Type of cases	No.of cases	Percentage
Tuberculoid type	11	24.44
Borderline tuberculoid	5	11.12
Borderline	11	24.44
Borderline lepromatous	5	11.12
Lepromatous	13	28.88
TOTAL	45	100.00

In control group age varied between 15-50 years with mean (±SE) of 30.07 ± 2.63 and in study group between 18 to 75 years with mean (± SE) of 39.64 ± 2.09. Out of total number of 45 cases study group included 28 males and 17 females. In control group there were 13 males and 2 females. The mean age (± SE) of male and female volunteers in control group was 30.07 ± 2.63 and 30.0 ± 4.96 respectively and in study group 39.42 ± 2.73 and 40.29 ± 3.47 respectively (Table III).

TABLE III

AGE AND SEX DISTRIBUTION OF CASES

Age range in years	Control group			Study group		
	Male	Female	Total	Male	Female	Total
11 - 20	2	-	2	3	-	3
21 - 30	4	1	5	7	4	11
31 - 40	6	1	7	7	6	13
41 - 50	1	-	1	5	3	8
51- 60	-	-	-	3	3	6
61 - 70	-	-	-	2	-	2
71 - 80	-	-	-	1	1	2
TOTAL	13	2	15	28	17	45
Mean ± SE	30.07 ± 2.63	30.00 ± 4.96	30.06 ± 2.36	39.42 ± 2.73	40.29 ± 3.47	39.64 ± 2.09

Age distribution in different types of leprosy shows that most of the cases were in the age range of 21-40 years. The mean age (± SE) in different types of leprosy is shown Table IV. The mean age (± SE) in tuberculoid leprosy (TT) was 32.18 ± 2.76, in borderline tuberculoid (BT) 27.40 ± 3.57, in borderline (BB) 50.0 ± 3.53, in borderline lepromatous (BL) 39.2 ± 2.92 and in lepromatous leprosy (LL) 43.60 ± 4.81. The average age of whole group was 39.64 ± 2.09 as stated before.

TABLE IV

AGE DISTRIBUTION OF DIFFERENT TYPES OF CASES

Age range in years	TT	BT	BB	BL	LL	Total
11 - 20	-	1	-	-	2	3
21 - 30	6	3	1	-	1	11
31 - 40	3	1	3	3	4	14
41 - 50	2	-	2	2	2	8
51 - 60	-	-	4	-	2	6
61 - 70	-	-	-	-	1	1
71 - 80	-	-	1	-	1	2
TOTAL	11	5	11	5	13	45
Mean ± SE	32.18 ± 2.76	27.40 ± 3.57	50.00 ± 3.53	39.20 ±2.92	43.60 ±4.81	39.64 ±2.09

In sex distribution of different types of leprosy, it was observed that in borderline tuberculoid type and lepromatous type there was male preponderance whereas other types revealed equal sex distribution. On percentage basis male preponderance has been found (Table V) i.e. 62.30% were males and only 37.70% females.

TABLE V

SEX DISTRIBUTION OF DIFFERENT TYPES OF CASES

Sex	TT	BT	BB	BL	LL	Total	Percentage
Male	6	4	5	2	11	28	62.30
Female	5	1	6	3	2	17	37.70
TOTAL	11	5	11	5	13	45	100.00

Certain prominent clinical manifestations of reactions observed in the 21 patients of leprosy are depicted in Table VI. The erythema nodosum leprosum was observed only in lepromatous and borderline lepromatous patient, not in tuberculoid and borderline patients. Neuritis was seen in the tuberculoid and borderline group while arthritis, iridocyclitis and lymphadenopathy were present among lepromatous patients.

TABLE VI

CLINICAL MANIFESTATION OF DIFFERENT CASES

Type of leprosy	Fever		Joint pain	Nerve pain thickening abcess	ENL	New lesion	Oedema of old lesion	Arthritis	Iridocyclisis	Lymphadenitis
TT	2	1	-	1	-	-	2	-	-	-
BT	-	-	-	-	-	-	-	-	-	-
BB	8	3	2	3	-	3	8	-	-	-
BL	1	1	-	-	-	-	1	-	-	-
LL	10	6	2	2	9	-	3	2	3	5

Clinically diagnosed 11 cases of tuberculoid type were found to be TT-5, BT-3, BB-2 and BL-1 histologically. 5 cases diagnosed as borderline tuberculoid clinically were found to be BT-2, BB-2 and BL-1 histologically. Clinically diagnosed 11 cases of Borderline were found histopathologically to be BB-6, BL-4 and LL-1. Clinically diagnosed 5 cases of borderline lepromatous were found to be BL-4 and LL-1 histopathologically. While clinically diagnosed 13 cases of lepromatous leprosy were confirmed histopathologically (Table VII).

TABLE VII

CLINICAL AND HISTOPATHOLOGICAL CORRELATION OF VARIOUS TYPES OF LEPROSY

Clinical		Histopathological				
Type of cases	No. of cases	TT	BT	BB	BL	LL
TT	11	5	3	2	1	-
BT	5	-	2	2	1	-
BB	11	-	-	6	4	1
BL	5	-	-	-	4	1
LL	13	-	-	-	-	13
TOTAL	45	5	5	10	10	15

Tuberculoid Leprosy

Two cases of tuberculoid leprosy showed reaction which was a reflection of exaggrated cell mediated immunity. The histologically features of this response could be recognised as follows :

(1) Intercellular and intracellular oedema. These changes are recognised as the cells appear swollen and vacuolated. Intereellular oedema produced spongy appearance.

(2) Infiltration by mononuclear cells.

(3) Degenerative changes were seen in mast cells with a decrease in their number.

Borderline group of leprosy

9 cases showed reactional changes. Two important features were seen beside the usual features of intracellular and intercellular oedema, polymorphonuclear and mononuclear infiltration and fibrinoid necrosis. Additional changes were :-

- Sub epidermal zone remained free in parts.
- In some of the lesions uninfiltrated and well preserved nerves were found.

Lepromatous leprosy

The histological features of lepromatous leprosy during reaction are termed as erythema nodosum leprosum (ENL). The feature consisted of :-

- Polymorphonuclear infiltration seen in 8 cases, beside this marked cellular disintegeration was also noted.
- Intracellular and intercellular oedema seen in 7 cases both in the dermis and epidermis.
- Fibrinoid necrosis of the blood vessel wall along with infiltration by polymorphs seen in 6 cases.
- Lepra bacilli were demonstrated in large numbers in these cases in the blood vessel wall (Table VIII).

TABLE VIII

HISTOPATHOLOGICAL CHANGES IN DIFFERENT TYPES OF LEPROSY SHOWING REACTIONS

Type of cases	No.of	Polymorphs & mononuclear	Oedema		Fibrinoid necrosis	Acid fast staining (AFS)
			Intracellular	Extracellular		
Tuberculoid type	2	2	2	-	-	-
Borderline tuberculoid	-	-	-	-	-	-
Borderline	8	8	3	3	2	3
Borderline lepromatous	1	1	1	-	1	-
Lepromatous	10	8	4	3	6	5
TOTAL	21	18	10	6	9	8

The absolute lymphocyte count and T cell count were done in control group and different types of leprosy (Table IX, Fig I). The study showed that in control group, absolute lymphocyte count ranged from 1566 to 3128 with mean ± SE of 2482.80 ± 215.20. The T cell percentage ranges from 45 to 65 with mean ± SE of 57.0 ± 1.7 whereas absolute T cell count ranged from 851 to 3315 with mean ± SE of 1435.0 ± 236.4.

In tuberculoid type of cases absolute lymphocyte count ranged from 1700 to 3900 with mean ± SE of 2236.0 ± 222.5. T cell percentage ranged from 42 to 72 with mean ± SE of 57.63 ± 2.51. The absolute T cell count ranged from 847 to 1950 with mean ± SE of 1202.0 ± 203.62.

In borderline tuberculoid type absolute lymphocyte count ranged from 1530 to 3995 with mean ± SE of 2350 ±446.95. The T cell percentage ranged from 48 to 64 with mean ± SE of 56.0 ± 2.83. The absolute T cell count was mean ± SE of 1358.0 ± 324.12 with a range of 734 to 2547.

In borderline type of cases absolute lymphocyte count ranged from 1540 to 3325 with mean ± SE of 2383.0 ± 200.08. The T cell percentage ranged from 38 to 60 with mean ± SE of 48.90 ± 2.35. The absolute T cell count ranged from 696 to 1928 with mean ± SE of 1179.54 ± 138.15.

In borderline lepromatous absolute lymphocyte count ranged from 1932 to 3270 with mean ± SE of 2550.2 ± 394.93. T cell percentage ranged from 38 to 58 with mean± SE

of 50.40 ± 3.37. Absolute T cell count ranged from 996 to 1700 with mean ± SE of 1287.80 ± 168.95.

In lepromatous type of cases absolute lymphocyte count ranged from 1200 to 4048 with mean ± SE of 2729.07 ± 203.87. T cell percentage ranged from 26 to 69 with mean ± SE of 42.0 ± 3.20. Absolute T cell count ranged from 582 to 8820 with mean ± SE of 1748.84 ± 599.37.

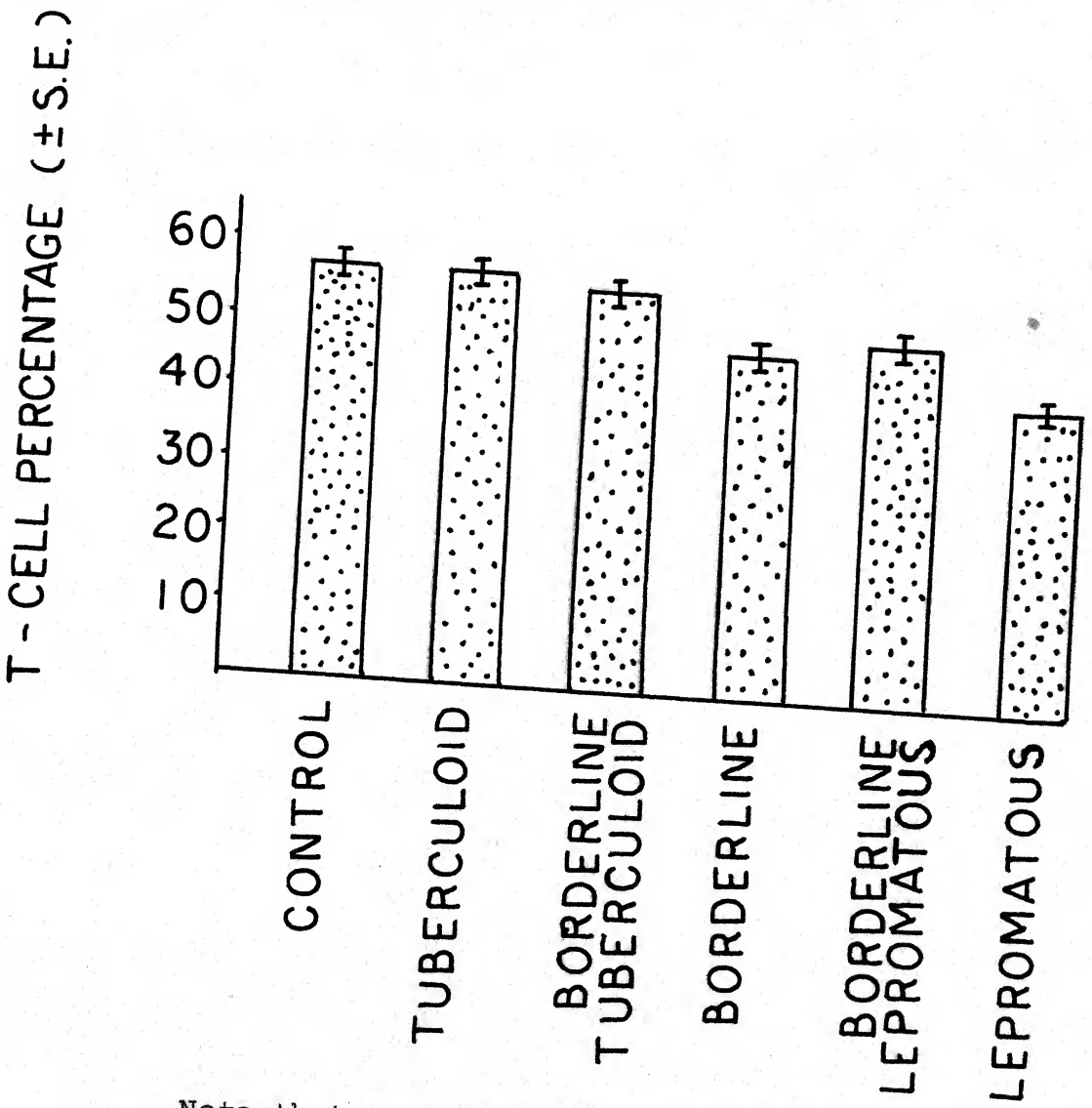
TABLE IX

DISTRIBUTION OF ABSOLUTE LYMPHOCYTE AND T CELL COUNT IN DIFFERENT TYPES OF LEPROSY AND CONTROL CASES

Types of cases (No.)	Absolute lymphocyte count(Mean±SE)	T cell percentage (Mean ± SE)	Absolute T cell count (Mean ± SE)
Control (15)	2482.8 ±215.20	57.00 ± 1.7	1435.0±236.4
Tuberculoid (11)	2236.0 ±222.05	47.63 ± 2.51	1202.0±203.62
Borderline tuberculoid (5)	2350.0 ±446.95	56.00 ± 2.83	1358.0±324.12
Borderline (11)	2383.0 ±200.08	48.90 ± 2.35	1179.54±138.15
Borderline lepromatous (05)	2550.2± 304.93	50.4 ± 3.37	1287.8±168.95
Lepromatous (13)	2729.07 ±203.87	42.0 ± 3.20	1748.84±599.37

FIG. 1.

T-CELL PERCENTAGE IN LEPROSY AND CONTROL CASES

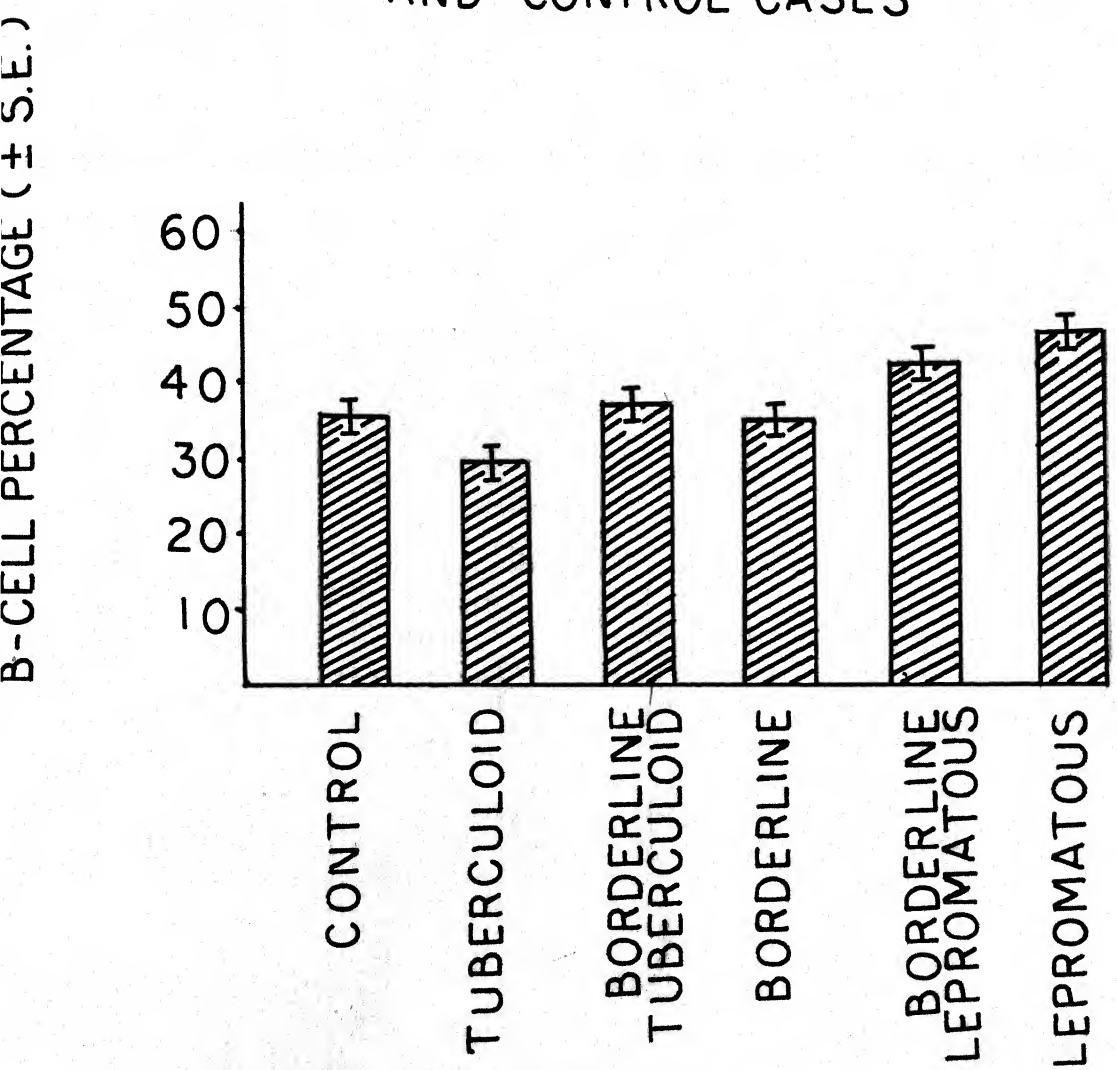


Note that mean T cell percentage gradually declined from tuberculoid to lepromatous pole.

FIG. 2.

B- CELL PERCENTAGE IN LEPROSY

AND CONTROL CASES

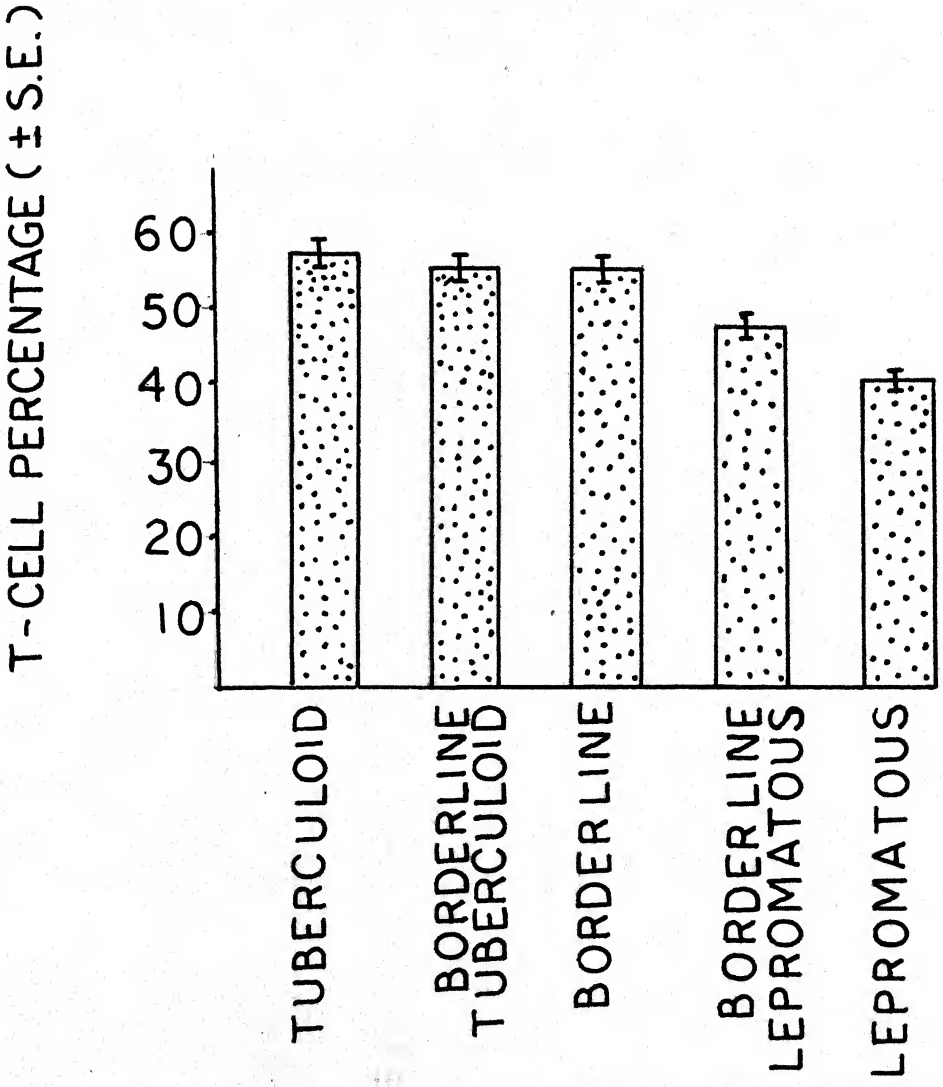


Note that contrary to mean T cell count change, B cell percent gradually increased from tuberculoid to lepromatous pole.

FIG. 3

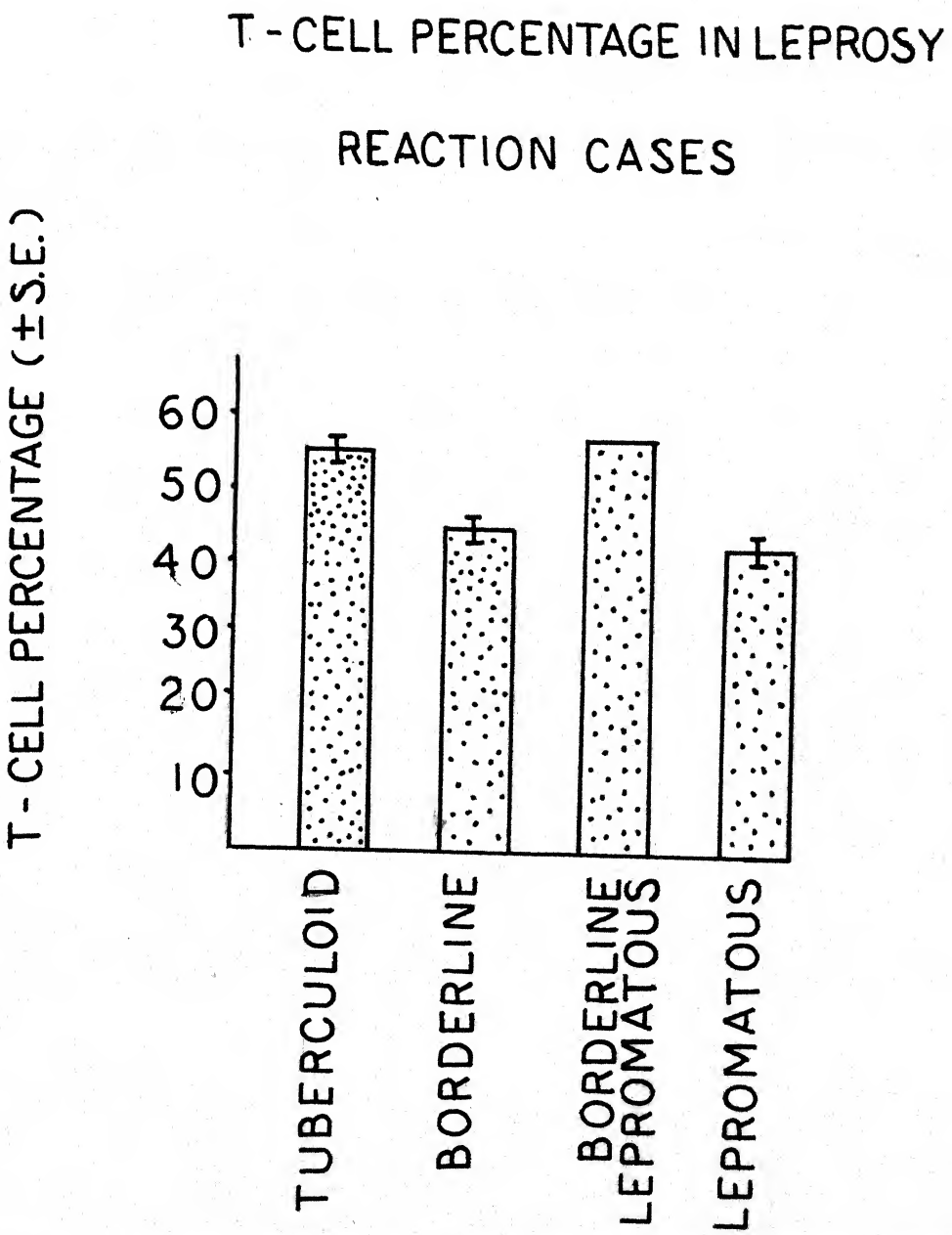
T - CELL PERCENTAGE IN LEPROSY

NON REACTION CASES



Note that in leprosy cases without reaction mean T cell percentage gradually declined from tuberculoid to lepromatous pole.

FIG. 4

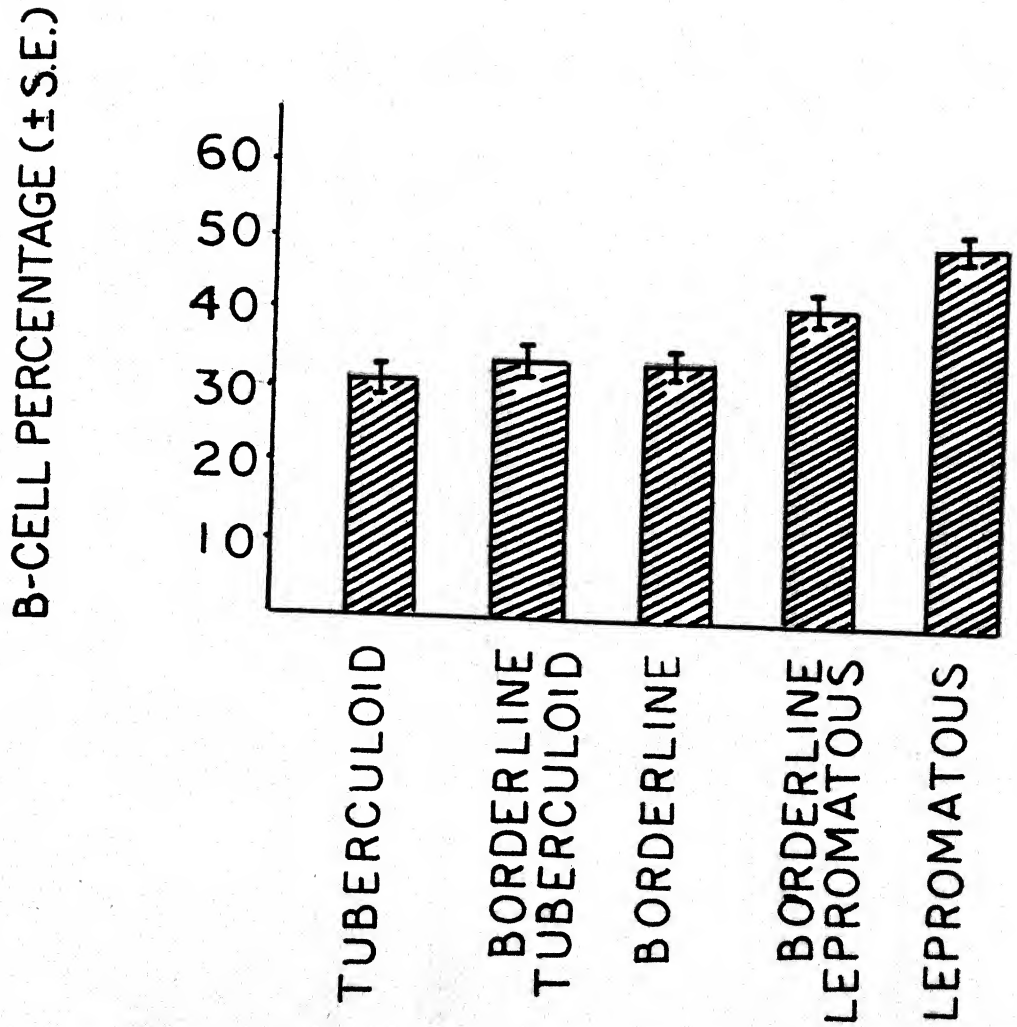


Note that in leprosy cases with reactions mean T cell percentage gradually declined from tuberculoid to lepromatous pole. There was only one case of borderline lepromatous leprosy in which T cell count was apparently high probably falling at the extreme of normal distribution curve and mean as well statistical significance could not be calculated.

FIG. 5

B-CELL PERCENTAGE IN LEPROSY

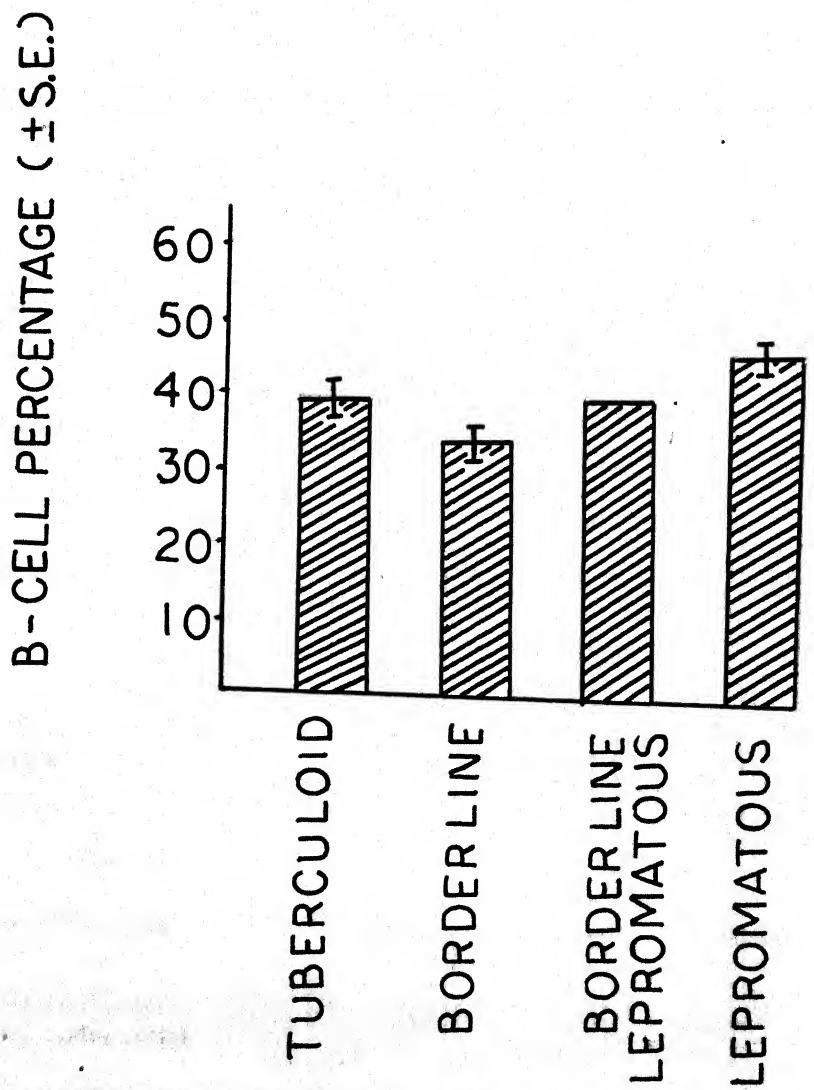
NON REACTION CASES.



Note that mean B cell percentage gradually increased from tuberculoid to lepromatous pole of leprosy cases without reaction.

FIG. 6

B- CELL PERCENTAGE IN LEPROSY REACTION CASES



Note that in leprosy cases with reaction mean B cell percentage increased from tuberculoid to lepromatous pole. Mean percent of B cell in borderline leprosy though was lesser than tuberculoid, it was not statistically significant. In borderline lepromatous there was only one case and mean and standard error of mean could not be calculated.

Significance of T cell percent between different groups is shown in Table X. T cell percentage has been found to be very highly significant ($p < 0.001$) in total leprosy cases when compared with control. The significance in difference of T cell percentage was also calculated between control and different types of leprosy cases separately.

TABLE X

SIGNIFICANCE OF T CELL PERCENTAGE BETWEEN DIFFERENT GROUPS

Type of cases	No.of cases	T cell % (Mean±SE)	Statistical significance			
			Difference between groups	'P' value	Difference between groups	'P' value
Control	15	57.0±1.66	-	-	-	-
Leprosy cases	45	50.0±1.58	Control & Leprosy cases	< 0.001	-	-
Tuberculoid type	11	57.63±2.51	C & TT	< 0.01	-	-
Borderline tuberculoid	05	56.0±2.83	C & BT	< 0.1	TT & BT	< 0.01
Borderline	11	48.90±2.35	C & BB	< 0.01	TT & BB	< 0.01
Borderline lepromatous	05	50.44±3.37	C & BL	< 0.01	TT & BL	< 0.01
Lepromatous	13	42.00±3.20	C & LL	< 0.001	TT & LL	< 0.001

SE = Standard error of mean

p < 0.01 Highly significant p < 0.001 Very highly significant

It has been observed that T cell percentage was highly significant ($p < 0.01$) in all types. T cell percentage gradually declined from TT to LL. The difference in T cell percentage between TT and BT, BB, TT and BL, TT and LL has been calculated and data obtained exhibited statistically very highly significant 'p' value (< 0.001) in T cell percentage.

Finding as observed for the status of T-lymphocyte population in various types of leprosy reaction cases are presented in Table XI.

TABLE XI

STATUS OF T-LYMPHOCYTE POPULATION IN VARIOUS TYPES OF LEPROSY REACTION CASES

Type of cases	No.of cases	Absolute lymphocyte count Mean±SE	Absolute T-cell count Mean±SE	T-cell percent Mean±SE
Tuberculoid type	2	1610±90.26	882.50±1.50	55.0±3.00
Borderline tuberculoid	-	-	-	-
Borderline	8	2479±194.04	1084.0±145.39	45.7±2.64
Borderline lepromatous	1	2925	1696	58
Lepromatous	10	2886±244.21	1183.0±124.07	43.50±3.98

In tuberculoid type of leprosy cases, absolute lymphocyte count ranged from 1520 to 1700 with mean ± SE of 1610 ± 90.26. Absolute T cell count ranged from 881 to 884 with mean ± SE of 882.50 ± 1.50, whereas T cell percentage ranged from 52 to 58 with Mean ± SE of 55.0 ± 3.00.

Only five patients had borderline tuberculoid type leprosy none of them had reaction. In borderline type, absolute lymphocyte count ranged from 1976 to 3325 with Mean ± SE of 2479 ± 194.04. Absolute T-cell count ranged from 699 to 1928 with Mean ± SE of 1084 ± 145.39. T-cell percentage ranged from 38 to 58 with Mean ± SE of 45.70 ± 2.64.

In borderline lepromatous type, absolute lymphocyte count calculated was 2925, absolute T-cell count 1696 and 58% of lymphocytes were T-cell.

In lepromatous type leprosy, absolute lymphocyte count ranged from 1600 to 4048 with Mean ± SE of 2886 ± 244.21. Absolute T-cell count ranged from 882 to 1657 with Mean ± SE of 1183 ± 124.07. T-cell percentage ranged from 28 to 69 with Mean ± SE of 43.5 ± 3.98 (Table XI, Fig IV).

In tuberculoid type of leprosy non-reaction cases absolute lymphocyte count ranged from 1700 to 3900 with Mean ± SE of 2375 ± 248.05. Absolute T-cell count ranged from 986 to 1950 with Mean ± SE of 1400 ± 201.78,

whereas T-cell percentage ranged from 42 to 72 with Mean ± SE of 58 ± 3.02. In borderline tuberculoid type non-reaction cases the absolute lymphocyte count ranged from 1530 to 3995 with Mean ± SE of 2350 ± 446.95. Absolute T-cell count ranged from 734 to 2547 with Mean ± SE of 1358 ± 324.14, whereas the T-cell percentage ranged from 48 to 64 with Mean ± SE of 56 ± 2.83. In borderline type non-reaction cases absolute lymphocyte count ranged from 1200 to 3280 with Mean ± SE of 2357 ± 593.94. T-cell count ranged from 696 to 1968 with Mean ± SE of 1313 ± 367.63 whereas T-cell percentage ranged from 50 to 60 with Mean ± SE of 56 ± 3.05. In borderline lepromatous type non-reaction cases the absolute lymphocyte count ranged from 1932 to 3270 with Mean ± SE of 2458 ± 312.69. The absolute T-cell count and percentage ranged from 966 to 1700 and 38 to 54 with Mean ± SE of 1185 ± 173.09 and 48 ± 3.59 respectively. In lepromatous type of non-reaction cases the absolute lymphocyte count ranged from 2375 to 2520 with Mean ± SE of 2432 ± 44.58. The absolute T-cell count and percentage ranged from 882 to 1428 and 34 to 54 respectively with Mean ± SE of 1197 ± 163.47 and 41 ± 6.50 respectively.

It was found that T-cell percentage in borderline leprosy non-reaction cases was significantly higher than borderline leprosy reaction cases ($p < 0.05$) (Table XII, Fig III).

TABLE XII

DISTRIBUTION OF ABSOLUTE LYMPHOCYTE AND T-CELL COUNT IN DIFFERENT TYPES OF LEPROSY NON REACTION CASES

Types of cases	No.of cases	Absolute lymphocyte count Mean ± SE	Absolute T-cell count Mean ± SE	T-cell percentage Mean ± SE	'p' value
Tuberculoid	9	2375±248.05	1440±201.78	58 ± 3.02	< 0.05
Borderline tuberculoid	5	2350±446.95	1358±324.14	56 ± 2.83	-
Borderline	3	2357±593.94	1313±367.63	56 ± 3.05	> 0.05
Borderline lepromatous	4	2458±312.69	1185±173.09	48 ± 3.59	-
Lepromatous	3	2432±44.58	1197±163.47	41 ± 6.50	< 0.05

'p' value compared with reaction cases.

TABLE XIII

DISTRIBUTION OF B CELL COUNT IN DIFFERENT TYPES OF LEPROSY AND CONTROL CASES

Types of cases	No.of cases	B cell percentage Mean ± SE	Absolute B cell count Mean ± SE
Control	15	36.46 ± 2.54	889.40 ± 80.58
Tuberculois type	11	30.54 ± 3.82	742.90 ± 72.35
Borderline tuberculoid	5	38.20 ± 5.65	783.40 ± 109.06
Borderline	11	36.90 ± 1.71	877.60 ± 77.69
Borderline lepromatous	5	44.20 ± 1.49	1135.00 ± 138.50
Lepromatous	13	48.38 ± 2.90	1388.61 ± 153.76

In control group B cell percentage ranged from 25 to 48 with Mean ±SE of 36.46 ± 2.54. Absolute B cell count ranged from 440 to 1530 with Mean ± SE of 889.40 ± 80.58. In tuberculoid type of cases B cell percentage ranged from 25 to 65 with Mean ± SE of 30.54 ± 3.82. The absolute B cell count ranged from 459 to 1248 with Mean ± SE of 742.90 ± 72.35.

In borderline tuberculoid type, B cell percentage ranged from 28 to 52 with Mean ± SE of 38.20 ± 5.65. Absolute B cell count ranged from 601 to 1198 with Mean±SE

of 783.40 ± 109.06. In borderline type, B-cell percentage ranged from 29 to 45 with Mean ± SE of 36.90 ± 1.71. Absolute B-cell count ranged from 348 to 1330 with Mean ± SE of 877.6 ± 77.69.

In borderline lepromatous type of cases, B-cell percentage ranged from 40 to 48 with Mean ± SE of 44.2 ± 1.49. Absolute B-cell count ranged from 772 to 1536 with Mean ± SE of 1135 ± 138.50.

In lepromatous type, B-cell percentage ranged from 31 to 65 with Mean ± SE of 48.38 ± 2.90. Absolute B-cell count ranged from 496 to 2629 with Mean ± SE of 1388.61 ± 153.76 (Table XIII, Fig II).

B-cell count in different types of leprosy has been compared with control to find out the significance of the difference. A marked difference in the mean of B-cell percentage of the control group and leprosy cases has been noted. Findings suggest that there is significant gradual increase in the mean percentage of B-cell from TT to LL. The statistical significance was calculated between control and leprosy cases and found to be very highly significant ($p < 0.001$). The comparison of control with TT, BT, BB, BL and LL whereas in cases of TT, BT and BB, it was significant ($p < 0.01$). The difference in B-cell percentage is also calculated between TT and BT, TT and BB, TT and BL, TT and LL and very highly significant ($p < 0.001$) values were obtained

except BT and BB, where it was significant only ($p < 0.01$). The above findings are shown in table XIV.

TABLE XIV

SIGNIFICANCE OF B-CELL PERCENT BETWEEN GROUPS

Type of cases	No.of cases studied	B-cell % Mean±SE	Statistical significance			
			Difference between groups	'P' value	Difference between groups	'P' value
Control (C)	15	36.46±2.49	-	-	-	-
Total leprosy cases	45	40.02±1.51	Control & Total Leprosy	< 0.001	-	-
TT	11	30.54±3.04	C & TT	< 0.01	-	-
BT	5	38.20±5.64	C & BT	< 0.05	TT & BT	< 0.01
BB	11	36.90±1.71	C & BB	< 0.05	TT & BB	< 0.01
BL	5	44.2±1.49	C & BL	< 0.001	TT & BL	< 0.001
LL	13	48.38±2.90	C & LL	< 0.001	TT & LL	< 0.001

SE = Standard error of mean

$p < 0.01$ Highly significant $p < 0.001$ Very highly significant

In tuberculoid type of leprosy cases, B-cell percentage ranged from 34 to 45 with mean ± SE of 40.0±5.01. Absolute B-cell count ranged from 765 to 5320 with mean ± SE of 3042.5 ± 228.42.

Borderline tuberculoid type of cases did not show any reaction.

In borderline type, B-cell pereentage ranged from 25 to 44 with mean ± SE of 35.14 ± 2.64. Absolute B-cell count ranged from 688 to 1330 with mean ± SE of 897.5 ± 74.49.

In borderline type, B-cell percentage was 42.1 whereas absolute B-cell count was found to be 1228.

In lepromatous type of cases, B-cell percentage ranged 31 to 45 with mean ± SE of 47.3 ± 3.36. Absolute B-cell count ranged from 496 to 2629 with mean ± SE of 1363 ± 159.87. (Table XV, Fig VI).

TABLE XV

DISTRIBUTION OF B-CELL COUNT IN DIFFERENT TYPES OF LEPROSY REACTION CASES.

Types of cases	No.of cases	B-cell percentage (Mean ± SE)	Absolute B-cell count (Mean±SE)
Tuberculoid	2	40.0 ± 5.01	3042.5 ± 228.42
Borderline tuberculoid	-	-	-
Borderline	8	35.14± 2.64	897.5 ± 74.49
Borderline lepromatous	1	42.1	1228
Lepromatous	10	47.3 ± 3.36	1363.0 ± 159.87

In tuberculoid type of leprosy non reaction cases absolute B-cell count ranged from 459 to 1248 with Mean ± SE of 763 ± 85.36 and B-cell percentage ranged from 25 to 50 with Mean ± SE of 32 ± 2.92. In borderline tuberculoid non-reaction cases absolute B-cell count ranged from 601 to 1198 with Mean ± SE of 783 ± 109.06 and B-cell percentage ranged from 28 to 52 with Mean ± SE of 35 ± 4.52. In borderline non reaction cases absolute B-cell count and percentage ranged from 1016 to 1180 and 29 to 40 with mean ± SE of 848 ± 254.73 and 35 ± 3.21 respectively. In borderline lepromatous non reaction cases the absolute B-cell count and percentage ranged from 772 to 1536 and 40 to 48 with Mean ± SE of 111 ± 175.78 and 43 ± 1.70 respectively.

TABLE XVI

DISTRIBUTION OF ABSOLUTE LYMPHOCYTE AND B-CELL COUNT IN DIFFERENT TYPES OF LEPROSY NON REACTION CASES

Type of cases	No.of cases	Absolute lymphocyte count Mean ± SE	Absolute B-cell count Mean ± SE	B-cell percentage Mean ± SE	'p' value
Tuberculoid	9	2375±248.05	763±85.36	32 ± 2.92	7 0.05
Borderline tuberculoid	5	2350±446.96	783±109.06	35 ± 4.52	-
Borderline	3	2340±609.54	848±254.73	35 ± 3.21	7 0.05
Borderline lepromatous	4	2456±312.69	1111±175.78	43 ± 1.70	-
Lepromatous	3	2432±44.58	1274±185.24	52 ± 6.43	7 0.05

'p' value compared with reaction cases.

In lepromatous non reaction cases the absolute B-cell count and percentage ranged from 997 to 1625 and from 42 to 64 with Mean ± SE of 1247 ± 185.24 and 52 ±6.43 respectively.

No significant difference was observed between the B-cell distribution in reaction and non-reaction cases of leprosy ($p > 0.05$) (Table XVI, Fig V).

D I S C U S S I O N

DISCUSSION

It is well established that leprosy is an infectious disease caused by M. leprae. Despite this, majority of the people though exposed to infection do not show any signs and symptoms of leprosy and only a minor group is affected. This fact clearly indicates the difference in the immunological phenomenon in two groups of population. Previous studies by different workers have revealed that due to some unknown mechanism, majority of the population have good immunity against M. leprae and escape from clinical disease whereas others have low resistance. Recently, Mahadevan (1985) has proposed a hypothesis that genetic susceptibility is responsible for lepromatous type of disease. The concept is based on the idea that there is significant quantum of host pathogen interaction before the disease can manifest and clinically identified. In view of interaction between the phagocytic cell and susceptible individuals live M. leprae leads to negative modulation of immune competence of the susceptible individuals. Such negative modulation is mediated through structural alterations of infected cells and production of soluble factors that immuno-modulates the response of the host to the pathogens.

During the chronic course of the disease, however, in some cases, there is a sudden spurt of disease activity, quite different from the normal progression and this is termed reaction. The reactions are very common in different types of leprosy. The exact mechanism of reaction is not definite but seems to be immunological. The type I reactions occur because of changes in cell mediated immunity (CMI) (Godal et al., 1973; Barnetson, 1976), while type II reactions probably occur because of immune complexes (Bedi and Bhutani, 1975).

The present study was undertaken to study the status of T and B lymphocytes in different types of leprosy patients with and without reactions. The study included 60 subjects in total. Out of which 15 were normal healthy volunteers (relations of patients or hospital employees) forming the control group and 45 cases of different types of leprosy both with reaction and without reactions. The leprosy cases were classified according to Ridley and Jopling's (1966) classification. The maximum patients were clinically of the lepromatous group (28.88%), Borderline type (24.44%) and Tuberculoid type (24.44%) (Table II).

Out of 45 leprosy cases 62.30% were males while 37.70% were females. This may be because males are more exposed to the external environment thus infection and reinfection are common. The majority of males in the sample

suffered from lepromatous leprosy while majority of females had borderline leprosy (Table V). The maximum patients were in the age range of 21 years to 40 years (Table II and IV). About 50% cases of lepromatous leprosy suffered from fever. Out of 45 cases, 13 cases diagnosed clinically as lepromatous leprosy were confirmed histopathologically while in tuberculoid type 11 cases were diagnosed clinically but only 5 were proved so histopathologically. The difference or the lack of correlation between the clinical type of disease and histologically described variety of the disease is understandable. This difference may occur because of the fact that tuberculoid group included cases with TT or BT type of clinical picture. In some of the BT cases during reactions the histological picture may mimic the BL or BB. There is an obvious overlap in the borderline group (BT, BB and BL) as these are not well defined type of disease but a part continuous spectrum (Ridley and Jopling, 1962). Further, different types of lesions can occur simultaneously in a patient (Ganpati and Desikan, 1974). More lymphocytes were observed in tuberculoid lesions with reaction than those without reaction. There was also presence of oedema. In BB and BL patients with reactions there was also marked oedema of dermal connective tissue. The cells were polymorphs as well as lymphocytes. A free subepidermal zone was observed in two patients of BB leprosy with reaction. Similar clear subepidermal zone has been described by Skinsnes (1973).

In the present study follow up could not be done following the subsidence of reactions, neither lepromin test was performed during, before or after the reaction. Thus it is very difficult to say that type I reactions observed in this study were of down grading or reversal type. Influx of lymphocytes in the lesion during type I reactions has been described (Ridley, 1969). However, number of these infiltrating lymphocytes subsequently decrease in down grading reactions and the granuloma opens up much more. In contrast, though the number of infiltrating lymphocytes may decrease the granuloma becomes more compact with high number of epitheloid cells. Erythema nodosum leprosy (ENL) - type II reaction presents features of acute vasculitis often associated with fibrinoid swelling of collagen fibres with focal areas of necrosis with numerous inflammatory cells, predominantly polymorphonuclear. In our study, six out of nine LL patients with reactions, one BL case with reaction and two out of eight cases of BB type II reaction showed intracellular oedema as well as extracellular oedema along with fibrinoid necrosis. Evidence of vasculitis was present in all these cases. This is similar to what has been previously described (Bedi and Bhutani, 1975; Job et al., 1964; and Ridley, 1969).

Among the controls in the present study mean (±SE) absolute T cell count was 1435 ± 236.4 while the mean T cell percentage was 57 ± 1.7, this was little less than what has been reported by Rea et al. (1976). In their study mean T cell

percentage was 68.8 ± 7.7 however mean T cell percentage as reported by other workers has varied from 40.8 to 77 percent. (Dwyer et al, 1973; Lim et al, 1974; Chogle et al, 1977; Sharma et al, 1979). The differences in mean cell count among normal people have been studied by Mendes et al, (1974). Variation can occur because of the population difference and also in the donor when tested on different days. The reactive rossete formation of T cells and sheep red blood cells is temperature dependent. The maximum values are obtained between 10°C and 25°C and no rossete formation occurs at 37°C. Jhansi, the place of study is a warm dry area. The high temperature from March to July-August might have affected the cells during the transporation from the leprosy clinic to laboratory. Storage of blood and lymphocytes is known to affect T cell number. However, in the present study while comparing the data from controls and test subjects these factors are not applicable as climatic conditions are similar for both the groups. The minor and insignificant difference in the T cell count of this study and other studies may be due to above mentioned factors. The mean absolute lymphocyte count among the controls and different types of leprosy cases is depicted in Table XI. It is almost equal in all types of disease. This indicates that there is no correlation between the absolute lymphocyte count and the type of leprosy the patient has the mean T cell percentage among control was 57.00 ± 1.7, similar figures

were obtained for TT and BT group. The T cell percent was 48.90 ± 2.35 in BB, 50.4 ± 3.37 in BL and only 42.0 ± 3.20 among the lepromatous patients. Thus the mean T cells percentage was lowest among the lepromatous group when compared to control, the difference was highly significant. Similarly when compared to TT group the mean T cell percentage was very significantly low ($p < 0.001$). In other groups also the mean T cell percentage was significantly low when compared to controls, though the significance gradually declined from lowest values of lepromatous to maximum value of tuberculoid. Other workers have also reported similar findings showing the decrease in mean T cell percent from control to LL (Dwyer et al, 1973; Lim et al, 1974; Chogle et al, 1977; Kaklamanis et al, 1977). Recently Sharma et al, (1979) and Singh et al, (1983) made similar observations. These studies also indicated that there is gradual decrease in the mean T cell percent from control to LL group. In contrast to the above, no difference in mean T cell percent among the control and LL leprosy subjects was observed by Rea et al, (1976). They found mean T cell percent 70.4 ± 6.3 and 68.8 ± 7.7 among the controls and LL patients respectively. Our findings are further corroborated by Mendes et al, (1974). He studied T and B cells in peripheral blood and lymphnodes of lepromatous leprosy cases and found a significant decrease of T cells in the blood as well as the paracortical areas of

lymphnodes. Turk and Waters (1971) also made similar observation.

The mean B cell percentage (Table XIII) was 36.46 ±2.54 among the controls. Similar value was obtained for borderline cases and borderline tuberculoid cases. In the tuberculoid group the mean B cell percentage was only 30.54 ± 3.82 (range 34 to 45). The difference was significant in comparison to control. Contrary to this, B cell count was raised in borderline lepromatous and lepromatous patients 44.2 ± 1.49 and 48.38 ± 2.90 respectively. The increase in B cell count in comparison to control in both these groups was very highly significant ($p < 0.001$). The same was true when compared with tuberculoid group. The differences were again significant between tuberculoid and BT/BB groups with tuberculoid patients showing the lowest value as mentioned.

Gazl-Paczalska et al, (1973) had reported marked increase in B cell percentage 60% to 85% of B cell in peripheral blood. Sharma et al, (1979) had studied B cell percentage in complete spectrum of leprosy and observed slight increase in B cell percentages. They observed mean ± SE of B cell percentage in control, TT, BT, BB, BL and LL which was 27.67 ± 3.77, 28.50 ± 5.71, 30.23 ± 7.89, 31.85 ± 7.81 and 29.97 ± 7.79 respectively and concluded that there was very minimal increase in B cell percentage which was statistically not significant. Rea et al, (1976) had also observed no significant increase in B cells in lepromatous leprosy.

Dwyer et al, (1973) had studied B cell percentage only in control and lepromatous groups and found significant increase in B cell percentage in peripheral blood in lepromatous group. They observed 27 percent in control and 35 percent B cell in lepromatous group which are similar to the observations of present study. Other workers also had shown that patients with lepromatous leprosy had high proportion of circulating lymphocytes possessing membrane bound immunoglobulin (B cells). It has been proposed that such increase in B cell might represent over compensation for a deficiency of T lymphocytes.

The findings of present study were also in accordance with Chogle et al, (1977), who studied mean $\pm$ SE of B cell percentage in crontrol, TT, BB and LL groups and found 27 ± 5.4, 36 ± 8.6, 37 ± 6.6 and 56 ± 10.7 respectively.

The findings of present study are supported by the study of Verma et al, (1971) who had observed significant increase in B cell percentage among lymphocytes obtained from crushing the lymphnode.

As B-lymphocytes are involved in antibody production, Abe et al, (1972) had reported anti M. leprae antibodies with indirect fluorescent technique in both lepromatous, tuberculoid, and indeterminate sera but the proportion of positive sera titre observed was highest in lepromatous sera.

B cell percentage was confirmed between the leprosy patients with reaction and without reaction. There was no significant difference between these two groups, neither there was any correlation of mean B cell percentage during reaction to the type of disease patient had. This can be explained because of the fact that type I reactions occur due to changes in CMI (Bedi and Bhutani, 1975) and type II reaction which occur in multibacillary patients are immune complex mediated (Gelber et al, 1973). In the later group the humoral activity is already increased and various types of immunoglobulin sera have been demonstrated to be raised. (Uhr and Moller, 1968; Young Chaigud et al, 1975). Similarly the B cell count has also been demonstrated to be raised towards lepromatous pole than tuberculoid pole (Dwyer et al, 1973; Sharma et al, 1979; Sachdev et al, 1980).

The cell count was low among patients of all types with reactions when compared to patients without reactions (Table XI and XII). In the two tuberculoid patients it was 52% and 58% respectively (absolute T cell count 884 and 881). Among the borderline patients with reaction, three patients had T cell percent between 38 and 40% while two patients each had count of 48 and 50% respectively. Only one patient had count as high as 58% (absolute T cell count 1928). In contrast two reacting lepromatous patients had very low

T cell count that is 28% (absolute values 582, 1117 respectively). Other four patients had also low values between 35 and 41%. Three lepromatous patients with reaction had count around 50% and only one had high count about 60% (absolute value 1104).

The T cell count was not different among TT patients whether with reaction or without reaction. Though improvement of CMI is expected during reversal reaction, it is not necessary that T cell count in sera will increase. The changes in CMI are well documented by the influx of lymphocyte in lesions during reacting phase. The other parameters like lepromin test, blast transformation, macrophage inhibition studies were not done in this study. Further, patients could not be studied before, during and after the reactions, thus it is difficult to corroborate reversal nature of the reaction in these two tuberculoid patients.

The recent development of monoclonal antibodies had helped in identifying various phenotypes of T cells in leprosy infiltrates. This may distinguish different reaction states such as ENL from upgrading or borderline (Wesley-Mis et al, 1982).

The nature and histological pattern of the cutaneous infiltrates of 17 leprosy patients in reversal patient (Type I) and ENL, (Type II) were compared with tissue from 18 non reactional borderline leprosy (BT and BL) and lepromatous leprosy (LL) patients using monoclonal antibodies and

immunofluorescence. Reactional BT lesions showed a mild increase in OKT-11 + Pan T cells as compared to non reactional tissues and a significant influx of OKT-8 + (Suppressor/Cytotoxic) cells which were peripherally localised in the lymphocytes mantle sorrounding the epitheloid cells. The leu 3a + (helper inducer) cells were scattered amongst the lymphocytes and macrophages. The mean ratio ± SE of leu 3a +/OKT-8 + cells was 1.88 ± 0.64 in BT reactions as compared to 2.95 ± 0.95 in BT lesions. In contrast, lesions of BL reversal reactions and ENL showed a more marked increase in Pan T cells with a preponderance of helper/inducer subset, leu 3a +/OKT-8 + ratio being 2.26 ± 0.61 and 0.93 ± 0.57 in BL reactional and non reactional lesions, respectively. Interestingly, this increase in number of T cells reached levels observed in BT lesions. The distribution pattern of OKT-8 + cell was similar to leu 3a +, both being diffusely scattered on the bacilli laden macrophages. Ia like antigens were present in all granulomas and were abundant on lymphocytes and macrophases and less conspicous on epitheloid cells. T6 + langerhams cell were uniformly increased in all reactional lesions. It would appear that the changes observed in both type I and type II reactions are similar in the lepromatous group of patients. They differ significantly from the BT reversal reaction in terms of the dominant T cells subset and the microanatomical distribution of the OKT-8 + cells in the lesions (Narayana et al, 1983).

Similar observation was made from the borderline patients with reactions, also it was difficult to differentiate, nature of reaction whether reversal or downgrading.

All the ten lepromatous patients and sole BL patient with reaction had type II reaction. The low T cell count observed among these were comparable to those observed in patients without reactions. The course of ENL is proposed to be immuno-complex mediated mechanism (Wemambu et al,1969). It is akin to type III arthus reaction (Waters and Turk, 1971; Gelber, 1973). Demonstration of immune complexes in and around blood vessels is not universally found in ENL lesions (Faber et al, 1978). A cell mediated immune pathogenesis has also been proposed (Waldorf et al, 1966; Gream, 1971).

Mshana (1982) also proposed that ENL reaction is initiated by changes in the cell mediated immune reaction, that is, decrease in absolute or relative suppressor T cells. This is based on certain clinical observations, for example, not all LL patients develop ENL though all of them have high bacillary load and anti M. leprae antibodies. It has also recently been shown that, whereas, contact sensitivity to dinitro-chlorobenzene (DNBC) is depressed in lepromatous leprosy, it is, on the other hand, not impaired or greatly attenuated during ENL (Rea et al, 1980), indicating a depression of suppression. Depression of suppressor cell during

ENL with a concomitant increase in in-vitro phytohaemagglutinin (PHA) response has been demonstrated by Bach et al (1980). However, in-vivo responses to M.leprae were not affected in their studies. It is interesting to note that Wesley et al (1982) in his study was able to demonstrate only few OKT-4/leu - 3a cells, T cells in the infiltrate of patients with erythema nodosum leprosum, while the sole patient whose disease had changed from borderline to tuberculoid leprosy had large number of OKT-4 Len-za cells, similar to that found in tuberculoid lesion.

Keeping in view the work of other authors and the present study, one can establish that there is gradual depression of CMI among leprosy patients from the tuberculoid pole to lepromatous pole. This is evident from lepromin test, lymphocyte transformation test and cutaneous delayed hypersensitivity reaction. The borderline group bridges the two poles with varying intermediate results. The B cell percentage gradually increases from tuberculoid to lepromatous end. While T cells in the peripheral blood though normal in number among tuberculoid patients are significantly low in lepromatous group. T cell percentage was significantly low among BB and BL patients also but the fall in the number was much less than that in LL patients. No difference was observed in the number of B cells in blood from patients with reaction and without reaction. Similarly the T cell percentage was similar among TT patients with reaction and without reaction. However,

in contrast to this a low T cell count was observed among lepromatous (LL and BL) patients with reaction in comparison to those who did not have reaction. The difference was not significant. Among the borderline patients with reaction highly varied T cell count was observed in the blood, in some significantly low and in others equal to that seen among borderline patients without reaction. However, it was never high. Thus, we can say that T cell count alone does not affect the nature of reaction, and other parameters for assessing immunity should be carried out.

Modlin et al,(1985) also observed statistically fewer cells of the suppressor cytotoxic pheno type and a greater number of cells of the helper induced phenotype in patients with ENL as compared with those without ENL. Patients without ENL had a tissue helper suppressor ratio of 0.6 to 0.1 as compared with those with ENL, whose ratio was 2.1 $\pm$ 0.4. Prior therapy had no affect on the results. Their work further supports that cell mediated immuno response is important in the pathogenesis of ENL, either directly or by permitting production of the antibody critical to the formation of immune complex. They also observed a lack of relationship between tissue and peripheral blood helper suppressor ratio. This finding is consistent with a process of selectivity of entry, retention or exit, or some combination of these rather than a passive diffusion of lymphocytes from the vascular component of tissues.

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

Leprosy does not only handicap a person physically but it does hurt the sufferer socio-economically and psychologically. It is widely distributed in all parts of the world and in India an estimated 4.0 million people suffer from leprosy. Traditionally leprosy is classified into three subtypes i.e. Tuberculoid (TT), Borderline (BB) and Lepromatous (LL). Ridley and Jopling (1966) added two intermediary categories e.g. borderline with tuberculoid features (BT) and borderline with lepromatous features (BL). Thus, TT, BT, BB, BL, LL comprise a spectrum in continuity. TT patients have highest immunity and other groups have immunity in descending order with lowest in LL patients.

In the present study, cellular immunity was assessed in different types of leprosy patients with estimation of T and B cells in peripheral blood. Along with this, the status of T and B cells in leprosy patients with and without reaction has also been studied as leprosy reaction (acute spurt of chronic disease) is supposed to have an immunological background.

The studies have been conducted on patients suffering from various types of leprosy, admitted in the ward or attending the out patients department of skin and VD. A thorough clinical examination was done and diagnosis

was established by biopsy. All the cases were classified according to Ridley and Jopling (1966) classification into five groups as mentioned above. The T and B lymphocyte counts have been done using E-rosette and EAC rosette techniques T and B lymphocyte percentage have been calculated from total and differential counts.

There is significant quantum of host pathogen interaction (pertaining to immunity) before disease is manifested into different subtypes. The sudden spurt of disease activity during chronic course termed as 'reaction' has also immunological basis. The leprosy cases have been further divided into non reaction and reaction cases. The status of T and B lymphocytes was seen in these two groups with different types of leprosy.

In this study 60 persons were included. Healthy fifteen formed the control group, and forty five were the patients with various types of leprosy. The maximum number of patients had lepromatous (28.88%) type of leprosy followed by tuberculoid (24.44%) and borderline (24.44%) types of leprosy. The maximum number of patients were between the age of 21 and 40 years and were predominantly male. This is because the lepromatous type of leprosy is highly infective variety and males are very often exposed to external environment falling prey to infection.

In contrast to T cell, B cell percentage gradually increased from TT to LL type. The difference in B cell percentage between different groups is significant. The B cell percent between reaction and non reaction cases were compared and no statistically significant difference was noted.

It is concluded from the present study that Thymus dependent lymphocytes (T cells) which are responsible for cell mediated immunity are decreased in leprosy in comparison to normal individuals. Conversely, Bursa dependent lymphocytes (so called B cells) which are responsible for humoral immunity are increased in leprosy. Furthermore, T cells show a gradual decrease as the disease progresses from tuberculoid to lepromatous stage. The B-cell count shows opposite changes. Excepting borderline, apparently no change was observed in T and B cell status in leprosy cases with reaction as compared to those who had no reaction. However, this conclusion may not be correct in absolute terms. It is presumed that downgrading reactions and upgrading reactions having opposite immunological status should have opposing influence on T cells. In the present study, reaction cases were not studied separately as upgrading and downgrading reactions and hence any change in T cell status in different reaction types, even if existing, was nullified.

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A P P E N D I X

APPENDIX I

STUDY OF 'T' AND 'B' CELLS IN LEPROSY PATIENTS WITH AND WITHOUT REACTIONS

INVESTIGATOR : Dr. RITA SAXENA

GUIDE : Dr. V.P. MITAL, M.D.

CO-GUIDE : Dr. D.C.Govil, M.D, DVD

1. Sl.No. _____ Case/MRD No. _____ Date _____

2. Name of Patient _____ Age/Sex _____

3. Address _____

4. Marital status _____ Married/Single

5. Occupation : House wife/Labourer/Office worker/Student/ Farmer

6. Socioeconomic status : Class I II III IV V

7. Rural/Urban

8. H.O.P.I.

(a) Numbness (b) Skin manifestation (c) Anaesthesia

(d) Deformities (e) Age of onset (f) Fever

Reaction present Mild/Moderate/Severe

Absent

(g) Duration of present reaction in days :

(i) 1-2 (ii) ∠ 5 (iii) 6-10 (iv) 11-25 (v) 26-50 (vi) 7 50.

9. Past history

(a) Tuberculosis (b) Syphilis (c) Other diseases

(d) Reaction (i) Type I/II

(ii) Total No. of reaction till now _____

(iii) Duration of occurrence of reaction- Recurring/ Persistent

(iv) Treatment in past

	Duration	Date	Regularty
Dapsone			
Rifampicin			
Clofazimine			
Corticosteroids			
Aspirin			
Chloroquine			

10. Family history : Tuberculosis/Leprosy

11. Personal history :

(i) Vegetarian/Nonvegetarian (2) Alcoholic/Nonalcoholic

(iii) Smoker/Non-smoker (4) Vaccination

12. General examination :

(a) Look (b) Build (c) PR (d) RR (e) BB mm of hg (f) Anaemia (g) Cyanosis (h) Jaundice

(i) Clubbing (j) Lymphnode (k) Eyes (l) Ear

(m) Eyebrows (n) Oedema : Face/Feet/Abdomen/Generalized

(o) Liver (p) Spleen

13. Systemic examination :

(a) Resp. System (b) C.V.S. (c) C.N.S.

INVESTIGATIONS

14. Blood Hb __ gm% ESR__ mm for Ist hour

T.L.C. ____ /Cu mm D.L.C.: P__ %, L __ %, E__ %, M __ %

G.B.P._____ Blood group _____

15. Skin biopsy : Histopath No._____ Report_____

AFS _____

16. Nasal smear _____ Skin smear _____

17. Histological findings

- Skin
- Other tissue
- A.F.S.

18. Absolute lymphocyte count

T-cell count T-cell %

B-cell count B-cell %

S U M M A R Y

SUMMARY AND CONCLUSION

Leprosy does not only handicap a person physically but it does hurt the sufferer socio-economically and psychologically. It is widely distributed in all parts of the world and in India an estimated 4.0 million people suffer from leprosy. Traditionally leprosy is classified into three subtypes i.e. Tuberculoid (TT), Borderline (BB) and Lepromatous (LL). Ridley and Jopling (1966) added two intermediary categories e.g. borderline with tuberculoid features (BT) and borderline with lepromatous features(BL). Thus TT, BT, BB, BL, LL comprise a spectrum in continuity. TT patients have highest immunity and other groups have immunity in descending order with lowest in LL patients.

In the present study, cellular immunity was assessed in different types of leprosy patients with estimation of T and B cells in peripheral blood. Along with this, the status of T and B cells in leprosy patients with and without reaction has been studied as leprosy reaction (acute spurt of chronic disease) is supposed to have an immunological background.

The studies have been conducted on patients suffering from various types of leprosy, admitted in the ward or attending the out patients department of Skin and VD. A thorough clinical examination was done and diagnosis

was established by biopsy. All the cases were classified according to Ridley and Jopling (1966) classification into five groups as mentioned above. The T and B lymphocyte counts have been done using E-rosette and EAC rosette techniques. T and B lymphocyte percentage have been calculated from total and differential counts.

There is significant quantum of host pathogen interaction (pertaining to immunity) before disease is manifested into different subtypes. The sudden spurt of disease activity during chronic course termed as 'reaction' has also immunological basis. The leprosy cases have been further divided into non reaction and reaction cases. The status of T and B lymphocytes was seen in these two groups with different types of leprosy.

In this study 60 persons were included. Healthy fifteen formed the control group, and forty five were the patients with various types of leprosy. The maximum number of patients had lepromatous (28.88%) type of leprosy followed by tuberculoid (24.44%) and borderline (24.44%) types of leprosy. The maximum number of patients were between the age of 21 and 40 years and were predominantly male. This is because the lepromatous type of leprosy is highly infective variety and males are very often exposed to external environment falling prey to infection.

About 50% of cases suffered from systemic manifestations as fever. Almost all (nine out of ten) cases of lepromatous type suffered from erythema nodosum leprosum.

All the clinically diagnosed lepromatous cases were confirmed histopathologically while in other types there was minor variation between clinical diagnosis and histopathological diagnosis. It was noted in this study that patients suffering from borderline or lepromatous type of leprosy may show any type of histopathological change pertaining to disease.

The T cell percent gradually declined from TT to LL type in comparison to control from 57.65 $\pm$ 2.51 to 42.0 $\pm$ 3.20. This is because of lower cell mediated immunity. The T cell percent in all leprosy cases is significantly lower than control cases. The T cell percent in control and TT type of leprosy is almost equal because of high degree of immunity in TT cases. From TT to LL types, the T cell percent gradually declined and the difference in T cell percent between these groups was statistically significant. It shows the presence of lower immunity status in descending order from TT to LL types.

T cell population in reaction and non reaction cases was also studied. In borderline non reaction cases the T cell percentage was significantly higher than reaction cases ($p < 0.05$).

In contrast to T cell, B cell percentage gradually increased from TT to LL type. The difference in the B cell percentage between different groups is significant. The B cell percent between reaction and non reaction cases were compared and no statistically significant difference was noted.

It is concluded from the present study that Thymus dependent lymphocytes (T cells) which are responsible for cell mediated immunity are decreased in leprosy in comparison to normal individuals. Conversely, Bursa dependent lymphocytes (so called B cells) which are responsible for humoral immunity are increased in leprosy. Furthermore, T cells show a gradual decrease as the disease progresses from tuberculoid to lepromatous stage. The B cell count shows opposite changes. Excepting borderline apparently no change was observed in T and B cell status in leprosy cases with reaction as compared to those who had no reaction. However, this conclusion may not be correct in absolute terms. It is presumed that downgrading reactions and upgrading reactions having opposite immunological status should have opposing influence on T cells. In the present study, reaction cases were not studied separately as upgrading and downgrading reactions and hence any change in T cell status in different reaction types, even if existing, was nullified.
